Iran Agricultural Research (2020) **39**(**2**) 69-80

Shiraz University

Research Article

Triticale forage crop quality as affected by water stress and nitrogen biofertilizer in an arid climate

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DOI: 10.22099/IAR.2021.38134.1404

ARTICLE INFO

***Article history*:**

Received **12 August 2020**

Accepted **4 January 2021**

Available online **30 January 2021**

***Keywords*:**

Azospirillum

Crude protein

Dry matter digestibility

Irrigation

ABSTRACT- Understanding the interactive effect of water and nitrogen (N) availability is a crucial issue for stabilizing cereal forage production in the arid areas. A two-year side by side experiment (2015-2016 and 2016-2017 growing seasons) was carried out under different N sources and water regimes in a typical arid environment (Darab, Iran) to evaluate the forage quality of triticale (×*Triticosecale* Wittmack) crop. There were two irrigation regimes [normal irrigation (IR_N) and cutting off irrigation after crop anthesis stage (IR_{MD})]. Rain-fed treatment (IR_0) was included in the second year. Three N sources including *Azospirillum brasilense* (biofertilizer, Bio), *Azospirillum brasilense* + 75 kg N ha^{-1} as urea (Bio + N_{75}), 150 kg N ha^{-1} as urea (N_{150}) and unfertilized plots (N_0 as control) were used. Water stress decreased leaf-stem (L/S) ratio, dry mater (DM) and dry matter digestibility (DMD) and increased crude forage protein (CP), neutral detergent fiber (NDF) and ash contents. However, the effect of water stress on forage ash, DMD, ADF contents, L/S ratio (in 2015-2016 growing season) and CP content depended on N sources. In Bio + N_{75}, triticale forage had the highest CP content, DMD, ash and L/S ratio (in 2015-2016 growing season) and the lowest ADF compared with the other N sources under water stress conditions. According to the results of this study, it can be suggested that the integration of biofertilizer and chemical N fertilizer can be successfully used to increase the quality of triticale forage under deficit irrigation regimes in sustainable farming systems in arid conditions.

INTRODUCTION

Triticale was intended as a bridge species to transfer the genetic material of rye (*Secale cereal* L.) to wheat (*Triticum aestivum* L.) to provide an alternative to wheat as a food crop. However, since the 1970s, there has been increased interest in utilization of triticale as a forage crop (Baron et al., 2015). Compared with the other cereals, triticale offers better amino acid balance, lysine content, and higher protein that are particularly important for swine (*Sus domesticus*) and poultry (Goverin et al., 2011). The increasing scarcity of irrigation water is the most important problem for forage production in arid and semi-arid environments; however, there is the potential to minimize agricultural water use by utilizing whole-plant winter cereals such as triticale for silage rather than relying on summer crops for feeding ruminants (Goverin et al., 2011). Furthermore, drought resistance of triticale has made it better adapted to forage legumes in Mediterranean climate (Droushiotis, 1985).

Although forage cereals crops are superior against the other forage crops in Mediterranean conditions, however, their forage quality negatively affect by late season high temperature and water stress that occur in these areas and these adverse effects drastically increase as a consequence of climate change in the future. Findings of Maleki Farahani and Chaichi (2013) showed that greater acid detergent fiber (ADF) and neutral detergent fiber (NDF) and lower dry-matter digestibility (DMD) and leaf-stem (L\S) ratio of barley (*Hordeum vulgare* L.) within a low input farming system in response to late season higher temperatures and water stress could cause a decline in forage quality of barley. Indeed, sustainable production of food and feed for an increasing world population is threatened by climate change, especially in arid areas with restricted production sources (Maleki Farahani and Chaichi, 2013).

Similarly, arid and semi-arid areas in which triticale can grow for forage production usually have low soil N fertility. Nitrogen nutrition has been found responsible for cereal yield loss in these areas (Anderson, 1985; Passioura, 2002). However, continuous and excessive N fertilizer application not only increases the production cost, but also contributes to pollution of environment mainly through volatilization and leaching (Dadrasan et al., 2015). Recently, application of biological N fertilizers has been recommended due to their lower ecological concerns compared with chemical N fertilizers and their successful crop production performances (Dadrasan et al., 2015).

Crop response to N fertilization depend on soil water availability (Barati et al., 2015). Therefore, understanding of water availability × N interaction effects, especially when slow release N sources such as bio-fertilizers are applied, is crucial importance for stabilizing the cereal production in arid regions. Indeed, finding the N fertilizing and water management options to fit crop requirements are necessary to sustainable use of N and water in low water availability areas.

It was hypothesized that inoculation of triticale seeds with *Azospirillum brasilense* bacteria as N bio-fertilizer can substitute in part for the use of chemical N fertilizer and will improve forage quality and quantity of triticale in response to water stress. Therefore, the response of triticale crop to N fertilization from chemical and biological sources was evaluated under different irrigation regimes in this study.

MATERIALS AND METHODS

Growth conditions

The experiment of this study in each year was conducted as a split plot pattern arranged in a randomized complete block design at the Experimental Farm of School of Agriculture and Natural Resources of Darab, Shiraz University (28° 50′ N, 54° 30′ E, and altitude 1180 m). The experiments were conducted during two consecutive winter growing seasons in 2015−2016 and 2016−2017, respectively. Climatic conditions of an agro-meteorological station near the experimental site are shown in Fig. 1. The physical and chemical properties of the soil are presented in Table 1.

Treatments consisted of two and three irrigation regimes in 2015-2016 and 2016-2017, respectively, and three types of N fertilizer management with three replications in both years. Main plots were assigned to irrigation regimes comprised normal irrigation [plots were normally irrigated until plants reached to the physiological maturity stage (ZGS95) (Zadocks et al., 1974), (IR_N)] and mild deficit irrigation [plots were normally irrigated only until plants reached to the full anthesis stage (ZGS69), (IR_{MD})] in both growing seasons. In 2016-2017, rain-fed treatment (IR_0) was included due to more precipitation forecasting. Sub plots were allocated to N fertilizers comprised of control [no N fertilizer (N_0)], sole chemical N fertilizer [150 kg N ha^{-1} was used as recommended N fertilizer, (N_{150})], sole biological fertilizer [seed inoculated with *Azospirillum brasilense*, (Bio)], and combined N fertilizer [50% chemical N fertilizer (75 kg N ha^{-1}) + seed inoculated by *Azospirillum brasilense*, (Bio + N_{75})].

In both growing seasons, *Azospirillum brasilense* was provided by the Soil and Water Research Institute, Karaj, Iran. A winter triticale cultivar (Sanabad) was selected based on well adaptation to arid areas and provided from Hasnabad station of Seed and Plant Improvement Institute (SPII), Karaj, Iran as well. Seeds were surface sterilized in 3% sodium hypochlorite (NaOCl) for 2 min and washed twice with sterile distilled water. Seeds were inoculated as previously described by Creus et al. (1996) and air dried in a laminar flow cabinet to 14% humidity, and stored for 2 days at 15 ºC in the dark until sown.

After a seasonal fallow, seed bed preparation included moldboard plowing to a depth of 25 to 30 cm and then two perpendicular light disking was conducted in October of 2015 and 2016. After seed bed preparation, the experimental site was divided into 24 and 36 plots in 2015-2016 and 2016-2017 growing seasons, respectively. Uniform triticale seeds were hand sown at a soil depth of approximately 2 cm on 11 December 2015 and 15 December 2016 in rows 20 cm apart giving 250 viable seeds m^{-2} in plots of 3 × 5 m. Adjacent plots were 1 m apart in each replication and a border of 5 m was established between the replicates to minimize N and water lateral movement. For surface furrow irrigation, dikes were established around the plots.

Table 1. Selected physical and chemical properties of the experimental field soil (0 – 30 cm) of this study

Soil properties	Units	2015-2016	2016-2017
Clay	%	18	18
Silt	%	44	44
Sand	%	38	38
Total N	%	0.12	0.10
Organic matter	%	1.88	1.84
EC	dS m^{-1}	1.07	1.08
pH	1:2 soil: water	7.44	7.42
Available P	mg kg^{-1}	21	19
Exchangeable K	mg kg^{-1}	339	341
Fe	mg kg^{-1}	5.61	5.66
Mn	mg kg^{-1}	16.00	16.01
Cu	mg kg^{-1}	1.66	1.54
Zn	mg kg^{-1}	0.65	0.63

Chemical N fertilizer (urea 46% N) was applied to each plot at three triticale developmental stages [tillering stage (ZGS21), beginning of stem elongation stage (ZGS31) and ear emergence stage (ZGS57)] in equal amounts. Because of no deficiency of the other nutrients including the K and P fertilizers (Table 1), they were not applied in soil. No herbicides and pesticides were applied throughout the growing seasons.

In both growing seasons, the plots with IR_N irrigation regime were normally irrigated until plants reached the physiological maturity stage (ZGS95). In contrast, the plots with IR_{MD} regime were normally irrigated until plants reached to full anthesis stage (ZGS69). Before each irrigation event, the soil profile was sampled to the 90-cm depth in 30-cm increments using a post-hole auger from 4 spots at the center of each plot in three replications. The volumetric water content of the soil layers was measured using the gravimetric method and the depth of irrigation was calculated using Equation (1):

$$D = \sum_{i=1}^{n}(\theta_{\text{fci}} - \theta_{\text{i}})\,\Delta Z_{\text{i}} \quad (1)$$

where D is the irrigation water depth (mm), i is equal to one layer, n is the number of soil layer, ∑ is summation of amount of irrigation water depth (mm) in n number of layer, θ_{fci} is volumetric water content at field capacity (cm^3 cm^{-3}) in the ith soil layer, θ_i is volumetric water content (cm^3 cm^{-3}) in the ith soil layer and ΔZi is the soil layer thickness (mm) in the ith soil layer.

Water was applied when the mean soil moisture of the plots dropped to less than 50% of the available moisture. The amount of water applied was calculated in terms of the water needed to refill 0 - 90 cm depth to field capacity and was measured by time-volume technique (Grimes et al., 1987; Barati and Ghadiri, 2017). Number of irrigation, total amount of irrigation water applied, rainfall amount and sum of amount of irrigation and rainfall during the growing seasons are presented in Table 2.

Measurements

At the soft dough stage (ZGS85), one-meter long sample were randomly harvested from the center of each plot and leaf-to-stem (L\S) ratio was measured. The samples were dried at 60 ºC for 72 h, and then dry matter (DM) was measured. The vegetative and reproductive portions were chopped into small pieces and mixed thoroughly. The mixed samples were ground to pass through a 1-mm sieve. The near infrared reflectance spectroscopy (Inframatic 8620 feed analyzer) (Perten, U. S.) method (Maleki Farahani and Chaichi, 2013) was used to assess the forage quality parameters (such as DMD, CP, ADF, ash and NDF).

Statistical Analysis

Due to the different irrigation regimes in 2015-2016 and 2016-2017, a separate analysis of variances (ANOVA) was used in each year. Statistical analyses were performed through the GLM procedure of version 9 of SAS (SAS, 2004) by using the correct error term to evaluate each factor and interaction. Least significant difference (LSD) at 0.05 probability level was used as mean separation test.

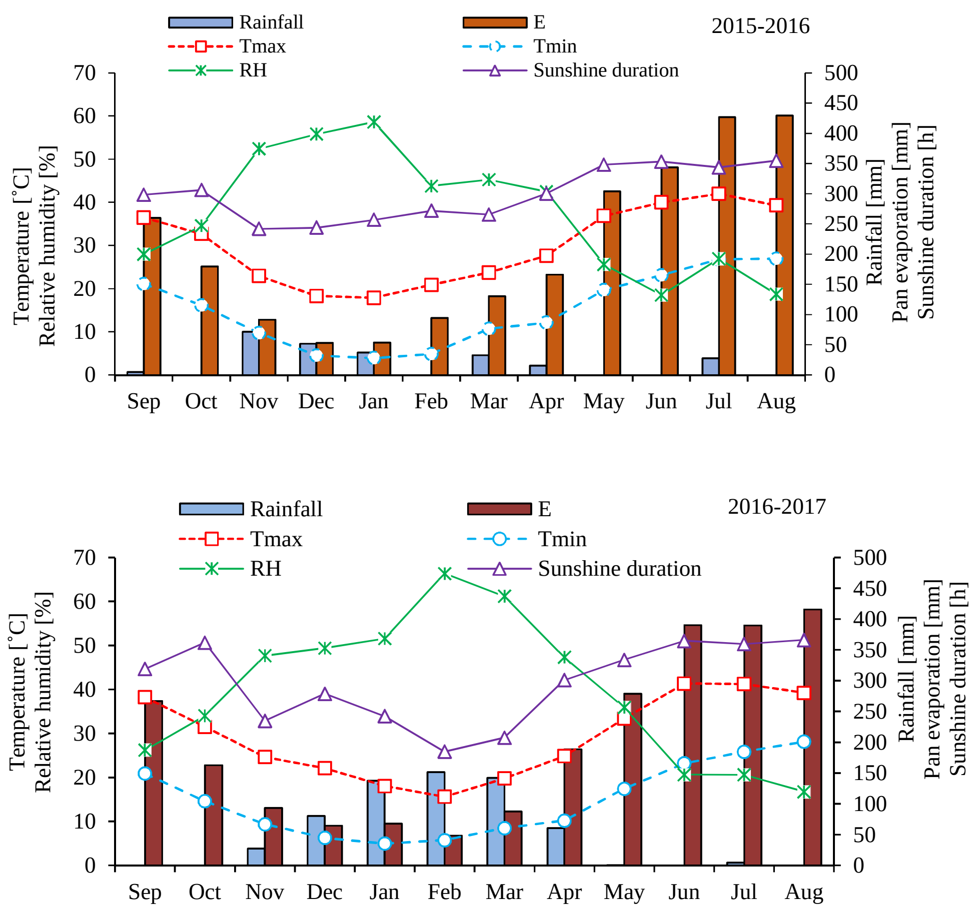

Fig. 1. Pan evaporation (E), monthly rainfall, mean minimum and maximum air temperatures (T_{min} and T_{max}, respectively), relative humidity (RH) and sunshine duration of an agro-meteorological station near the experimental site of this study during the two years of conducting experiments.

Table 2. Number of irrigation, total amount of irrigation water applied, rainfall amount and sum of amount of irrigation and rainfall during the growing seasons.

	2015-2016		2016-2017	
	IR_N	IR_{MD}	IR_N	IR_{MD}
Number of irrigation	10	7	5	2
Irrigation water applied [mm]	589.5	343.3	358.5	106.8
Rainfall amount [mm]	134.2	134.2	572.0	572.0
Sum of amount of irrigation and rainfall [mm]	723.7	477.5	930.5	678.8

IR_N: normal irrigation, IR_{MD}: mild deficit irrigation, IR_0: Rain fed

RESULTS

The leaf-to-stem (L/S) ratio of triticale varied from 0.19 to 0.35 over two growing seasons. Averaged across the normal and deficit irrigation (IR_N and IR_{MD}, respectively) treatments, L\S ratio of triticale crop was higher in 2016-2017 (0.31) than in 2015-2016 (0.27). In 2015-2016, IR_{MD} decreased L/S ratio in N_0 and N_{150} (1.7% and 24.7%, respectively). In contrast, IR_{MD} increased it in Bio and Bio + N_{75} treatments (13.7% and 4.1%, respectively) (significant irrigation × N fertilizer source interaction, Table 3) (Fig. 2). As a consequence, the highest L\S ratio (0.35) was achieved in Bio and IR_{MD} treatments and the lowest one (0.19) in N_0 and IR_{MD} treatments (Fig. 2). In 2016-2017, IR_{MD} slightly decreased L\S ratio (7.7%) compared with IR_N, however, it significantly declined (12.2%) with increasing water stress in rainfed conditions (IR_0) (Table 4). Nitrogen fertilizer significantly increased L\S ratio, however, there was no significant difference among the N sources (Table 4).

The mean dry matter (DM) was higher in 2016-2017 (10067 kg ha^{-1}) than that in 2015-2016 (9216 kg ha^{-1}). Irrespective of the N sources, N application significantly increased DM. The highest DM was achieved with Bio + N_{75} in 2015-2016 and by N_{150} in 2016-2017, while there was no significant difference in DM between integrated N fertilizer and sole chemical N fertilizer in both growing seasons (Table 4). The DM slightly decreased by IR_{MD} compared with IR_N in both growing seasons (3.4% and 4.3% in 1st and 2nd years of this study, respectively). But, increasing water stress by IR_0 treatment significantly decreased DM compared with IR_N (12%) (Table 4).

Across the 2-year period, the DMD was significantly affected by N fertilizing systems, irrigation regimes and their interactions (Table 3). The highest value of DMD (51.48 and 56 g kg^{-1} in 1st and 2nd years, respectively) belonged to Bio + $N_{75,}$ followed by sole biofertilizer (49.18 and 53.87 g kg^{-1} in 1st and 2nd years, respectively) under IR_N. Decreasing water supply by deficit irrigation treatments decreased DMD in all N sources, and the highest decrement of DMD was found in N_{150} (16.3% average over two years and 27.7% by IR_{MD} and IR_0, respectively) compared to the other N sources and the lowest amount of DMD belonged to Bio + N_{75} treatment, followed by Bio treatment (Fig. 3).

Nitrogen fertilizing systems and N fertilizer × irrigation interaction in both growing seasons significantly affected the ADF (Table 3). There was no significant difference among N sources under IR_N treatment. When crops exposed to water stress, the values of ADF in N_0 and especially N_{150} treatments showed increasing trend. In contrast, the values in Bio and Bio + N_{75} fertilizers had decreasing trend. Consequently, the highest value of ADF was achieved by N_{150} in IR_{MD} (in 2015-2016 and 2016-2017) and IR_0 (in 2016-2017) and the lowest value of ADF belonged to Bio and Bio + N_{75} fertilizers.

Water stress significantly affected NDF values in both growing seasons. It decreased 5.7% and 2.7% by IR_{MD} in first and second growing seasons, respectively compared with IR_N. In addition, the decreasing trend of NDF continued until reaching to 9.5% by IR_0 in the second growing season. There was no significant difference among N fertilizer treatments (N_{150}, Bio and Bio + N_{75}) in both growing seasons. However, regardless of the N sources, N fertilizer decreased NDF in the second year.

The forage crude protein (CP) was significantly influenced by all factors in both growing seasons (Table 3). Deficit irrigation increased CP, however, its increment was higher in N sources compared to the no N supply plots (IR × N interaction, P = 0.0727 and P = 0.0706 in 1st and 2nd year, respectively) (Fig. 4). The highest increment of CP as a consequence of deficit irrigation was occurred by Bio + N_{75} [23% by IR_{MD} (averaged over 2 years) and 30.8% by IR_0], followed by Bio and N_{150} in both years as well (Fig. 4).

The forage ash content was significantly affected by N fertilizer sources × irrigation regimes interaction in both growing seasons (Table 3). It had about constant values in N_0 and Bio treatments when exposed to water stress compared with IR_N conditions. However, in N_{150} treatment, the ash content followed a decreasing trend as water stress improved by IR_{MD} and IR_0 (Table 5). In contrast, IR_{MD} and IR_0 treatments significantly increased the ash content compared with IR_N when Bio + N_{75} treatment was applied (Table 5).

DISCUSSION

In arid environment of southern Iran, water limitation is a major constraint for crop production (Barati et al., 2015). In addition, continuous and excessive application of chemical N fertilizers not only contributes to environmental contaminations, but also increases production costs as well. Therefore, for better water and N fertilizer management in forage production, the whole forage triticale crop quality at the soft dough stage (ZGS85) under chemical and biological N fertilization and water deficit after anthesis was investigated in a 2-year research. The mean value of triticale L/S ratio in 2016-2017 (0.30) was higher than that in 2015-2016 (0.27) which can be attributed to higher rainfall in vegetative stage of the second year (572 mm) compared with the first year (86 mm) and lower relative humidity and higher temperature during the reproductive growth stage (March) in 2015-2016 as well (Fig. 1).

The L/S ratio had constant values or slightly increasing trend when water stress was imposed in biofertilizer (Bio and Bio + N_{75}) treatments. However, it sharply declined by consecutive drought stress treatments in N_{150} (Fig. 2).

In line with our results, several researchers (Day et al., 1987; Barati et al., 2015) showed that the application of sole chemical N fertilizer is the most beneficial for crop growth only if sufficient volume of irrigation water is applied. Furthermore, Maleki Farahani and Chaichi (2013) reported that the application of biofertilizers (P-solubilizing and N-fixing bacteria) alone and the supply of integrated chemical and biological N fertilizer were the most suitable options for achievement the highest L\S ratio under water stress conditions. In this study, higher L\S ratio that was observed in the Bio and Bio + N_{75} treatments compared with the N_{150} treatment under water stress conditions (IR_{MD} and IR_0) could be attributed to more dry mater remobilization from stem to grain in the Bio and Bio + N_{75} treatments than the N_{150} treatment (data not shown) and consequently more decrement of stem weight in Bio and Bio + N_{75} treatments than that in the N_{150} treatment under water stress conditions. Also, with respect to lower L\S ratio that was observed in N_{150} treatment under water stress conditions in this study, Barati and Ghadiri (2017) and Ercoli et al. (2008) demonstrated that when severe drought stress occurred, dry matter remobilization of barley and wheat was severely declined in high chemical N fertilizer application and considerable amount of non-structural carbohydrate remained in the stems. Therefore, L\S ratio can be reduced in this condition.

The dry matter (DM) of triticale crops was higher in 2016-2017 growing season, the growing season with higher water availability during the most sensitive stages of crops to water stress, compared with 2015-2016 growing season (Fig. 1 & Table 4). Indeed, response of crops to N fertilization is heavily reliant on rainfall distribution and availability of irrigation water (Tilling et al., 2007; Pala et al., 1996) and it is decreased during growing seasons with low precipitation (Rasmussen and Rohde, 1991). In this study, the DM was significantly decreased by severe drought stress (Table 4). In agreement with current results of this study, several authors including Shangguan et al. (2000); Wall et al. (2011); and Frederick and Camberato (1994) demonstrated that the leaf photosynthesis of barley and wheat decreased with increasing water stress. Therefore, depressing the photosynthesis rate during water stress period finally decrease DM. In this study, there was no significant difference in DM between integrated N fertilizer and sole chemical N fertilizer (Table 4). Therefore, with respect to ecological footprint of chemical N fertilizers compared with N biofertilizers and economic considerations, it is recommend to use integrated N fertilizer (75 kg chemical N + seed inoculation with *Azospirillum brasilense*) instead of other N sources to produce forage triticale in the environments having similar conditions of this study.

The DMD decreased when drought stress occurred, however, this decrement was different in the various N fertilizer sources (Fig. 3). The Bio and Bio + N_{75} treatments showed slightly reduction in DMD when exposed to water stress. In contrast, the crops that received N_{150} showed sharply and significantly reduction in DMD under water stress conditions in both growing seasons (Fig. 3). Therefore, the DMD response of forage triticale crop to water stress depends on the N fertilizer regimes and bio-fertilizers can diminish the adverse effect of water stress on triticale forage quality under unpredictable weather conditions such as the experimental conditions of this study. Several authors (Seligman and Sinclair, 1995; Safari et al., 2011) reported the decrease in DMD by drought stress. Also, Maleki farahani and Chaichi (2013) reported the superiority of bio-fertilizes under water stress conditions in DMD of forage barley. According to nutritional quality classification by Tainton (1999), forage containing digestible organic matter coefficients of more than 0.55, between 0.45-0.55, and less than 0.45 are of medium, low and poor nutritional quality, respectively. Therefore, in this study, N bio-fertilizer alone and integrated N fertilizer had medium nutritional values, whereas forages fertilized with chemical N fertilizer alone had poor digestibility, especially under drought stress after anthesis and rainfed conditions.

The ADF increased with consecutive drought stress in N_0 and especially in N_{150} in both growing seasons. By contrast, it decreased with biofertilizer alone and integrated N fertilizer applications when exposed to water stress conditions (Table 5). Maleki farahani and Chaichi (2013) reported that the decreased values of ADF in biofertilizer treatments under water stress conditions might be attributed to higher phosphorous (P) absorption in water scarcity environments. In addition, Brandt and Molgaared (2001) showed that ADF and plant P content had negative relationship. Indeed, the application of plant growth-promoting rhizobacteria (PGPR) as seed inoculants increased P uptake by enhancing crops water stress resistance through different mechanisms including improving root growth and development (Wu et al., 2013; Hardie and Leyton, 1981).

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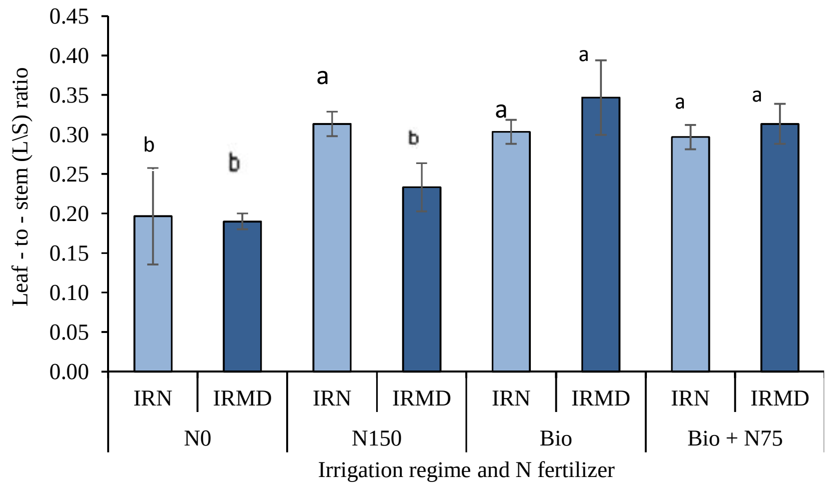
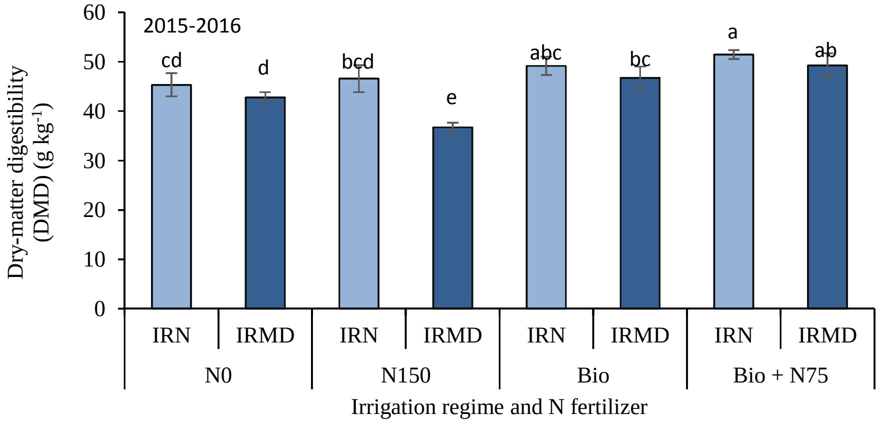


Fig. 2. Leaf-to-stem (L\S) ratio in relation to irrigation regimes and N fertilizer sources in 2015-2016 growing season. Error bars show standard deviation of the means. Bars with different letters have significant differences with each other according to the Fisher's LSD test ($P \leq 0.05$). LSD = 0.062. IR_N: normal irrigation, IR_{MD}: mild deficit irrigation.
N_0: no N fertilizer (control), N_{150}: sole chemical N fertilizer [150 kg N ha^{-1}], Bio: sole biological fertilizer (seed inoculated with *Azospirillum brasilense*), Bio + N_{75}: combined fertilizer (50% chemical N fertilizer [75 kg N ha^{-1}] + seed inoculated by *Azospirillum brasilense*).

Table 3. Mean squares for leaf-to-stem (L/S) ratio, dry matter (DM) , dry-matter digestibility (DMD), acid detergent fiber (ADF), neutral detergent fiber (NDF) and ash as influenced by irrigation regimes and N fertilizer sources in 2015-2016 and 2016-2017

Year	Source of variation	df	L/S	DM (kg ha^{-1})	DMD (g kg^{-1})	ADF (g kg^{-1})	NDF (g kg^{-1})	CP (%)	Ash (g kg^{-1})
2015-2016	Replication	2	0.00090^{NS}	36005.4^{NS}	0.13^{NS}	0.70^{NS}	1.31^{NS}	0.11^{NS}	0.1790^{NS}
	Irrigation (IR)	1	0.00027^{NS}	584064.2^{*}	109.40^{*}	0.66^{NS}	62.99^{*}	15.80^{*}	0.0009^{NS}
	E_a	2	0.00020	91349.2	1.12	3.01	3.02	0.34	0.1095
	N Fertilizer source (F)	3	0.02014^{**}	54340511.9^{**}	91.25^{**}	28.22^{**}	16.47^{NS}	13.05^{**}	2.1842^{**}
	IR × F	3	0.00421^{*}	435699.5^{NS}	21.24^{*}	16.10^{*}	0.33^{NS}	1.18^{NS}	08706^{*}
	CV [%]		12.66	10.83	4.79	5.41	6.23	5.39	7.05
2016-2017	Replication	2	0.00042^{NS}	447602.3^{NS}	0.23^{NS}	0.17^{NS}	0.70^{NS}	0.74^{NS}	0.1623^{NS}
	Irrigation (IR)	2	0.00421^{NS}	4479435.7^{*}	86.84^{*}	3.80^{NS}	83.05^{*}	17.20^{**}	0.1085^{NS}
	E_a	4	0.00062	246791.8	7.01	1.30	7.42	0.38	0.0684
	N Fertilizer source (F)	3	0.01151^{**}	77137330.9^{**}	360.17^{**}	176.23^{**}	44.49^{*}	24.74^{**}	5.9913^{**}
	IR × F	6	0.00114^{NS}	864945.4^{NS}	21.80^{*}	43.61^{**}	0.63^{NS}	1.52^{NS}	0.7284^{*}
	CV [%]		8.85	10.31	5.43	5.88	5.73	6.48	8.49

** Significant at $P \leq 0.01$, * significant at $P \leq 0.05$, and ^{NS} non-significant.



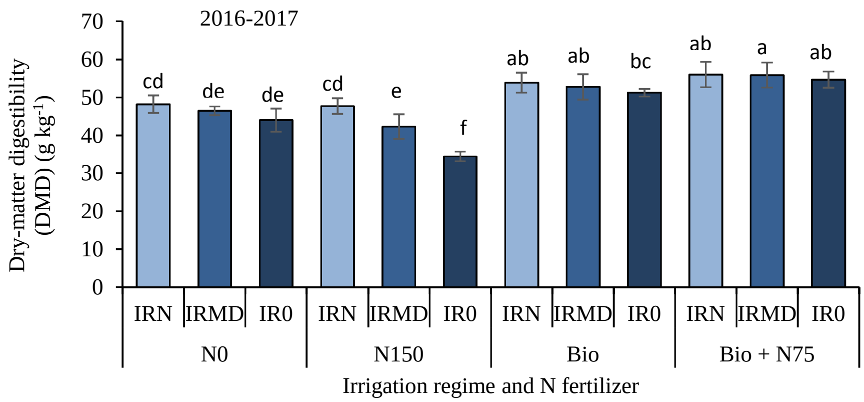
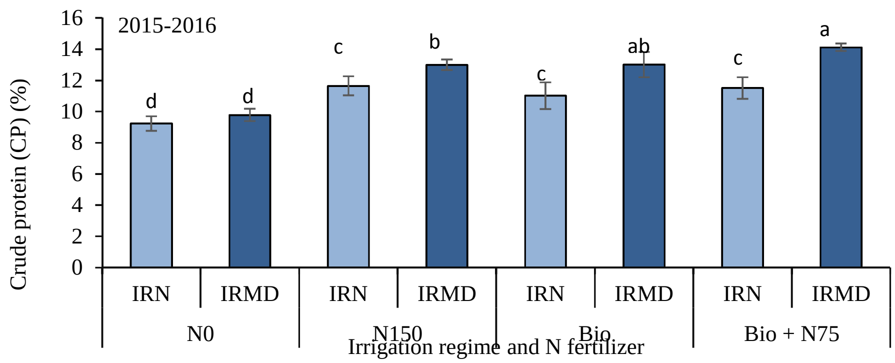


Fig. 3. Dry-matter digestibility (DMD) in relation to irrigation regimes and N fertilizer sources in both growing seasons. Error bars show standard deviation of the means. Bars with different letters have significant differences with each other according to the Fisher's LSD test ($P \leq 0.05$). LSD = 3.92 and 4.56 for 2015-2016 and 2016-2017, respectively.
IR_N: normal irrigation, IR_{MD}: mild deficit irrigation, IR_0: Rain fed.
N_0: no N fertilizer (control), N_{150}: sole chemical N fertilizer [150 kg N ha^{-1}], Bio: sole biological fertilizer (seed inoculated with *Azospirillum brasilense*), Bio + N_{75}: combined fertilizer (50% chemical N fertilizer [75 kg N ha^{-1}] + seed inoculated by *Azospirillum brasilense*).

Table 4. Leaf-to-stem (L/S) ratio, dry matter (DM) and neutral detergent fiber (NDF) as influenced by irrigation regimes and N fertilizer sources in 2015-2016 and 2016-2017.

Source of variation	L/S	DM (kg ha^{-1})		NDF (g kg^{-1})	
Irrigation	2016-2017	2015-2016	2016-2017	2015-2016	2016-2017
IR_N £	0.32^a	9172^a	10191^a	57.23^b	53.65^b
IR_{MD}	0.29^{ab}	8860^a	9744^a	60.47^a	55.12^b
IR_0	0.28^b	–	8971^b	–	58.76^a
LSD $_{(0.05)}$	0.03	531	563	3.05	3.09
Fertilizer					
N_0 §	0.24^b	4899^c	5398^c	56.94^a	56.68^{ab}
N_{150}	0.31^a	11216^a	11868^a	60.73^a	58.59^a
Bio	0.32^a	8644^b	9854^b	58.13^a	53.85^b
Bio + N_{75}	0.31^a	11306^a	11287^a	59.58^a	54.23^b
LSD $_{(0.05)}$	0.03	1228	981	4.62	3.17

Different letters are showing significant differences of the means according to the Fisher's LSD test ($P \leq 0.05$).
£ IR_N: normal irrigation, IR_{MD}: mild deficit irrigation, IR_0: Rainfed.
§ N_0: no N fertilizer (control), N_{150}: sole chemical N fertilizer [150 kg N ha^{-1}], Bio: sole biological fertilizer (seed inoculated with *Azospirillum brasilense*), Bio + N_{75}: combined fertilizer (50% chemical N fertilizer [75 kg N ha^{-1}] + seed inoculated by *Azospirillum brasilense*).



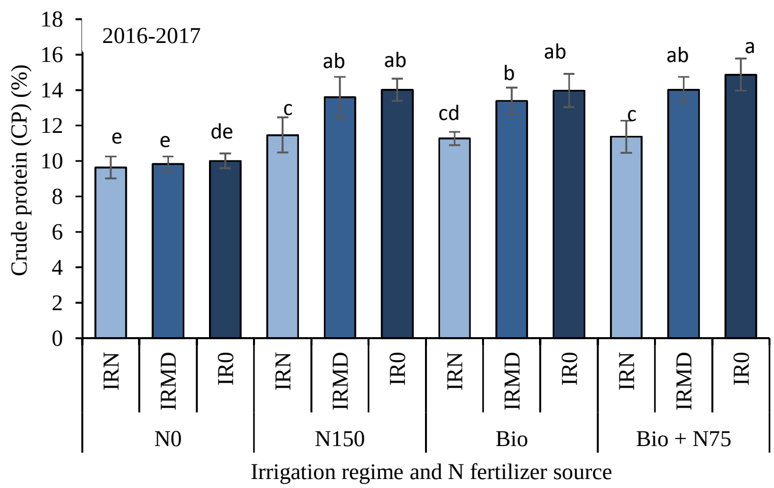


Fig. 4. Crude protein (CP) in relation to irrigation regimes and N fertilizer sources in both growing seasons. Error bars show standard deviation of the means. Bars with different letters have significant differences with each other according to the Fisher's LSD test ($P \leq 0.05$). LSD = 1.11 and 1.37 for 2015-2016 and 2016-2017, respectively.
IR_N: normal irrigation, IR_{MD}: mild deficit irrigation, IR_0: Rainfed.
N_0: no N fertilizer (control), N_{150}: sole chemical N fertilizer [150 kg N ha^{-1}], Bio: sole biological fertilizer (seed inoculated with *Azospirillum brasilense*), Bio + N_{75}: combined fertilizer (50% chemical N fertilizer [75 kg N ha^{-1}] + seed inoculated by *Azospirillum brasilense*).

In the present study, the NDF positively and significantly increased with successive water stress (Table 4). Increasing trend of NDF under drought stress conditions compared with ADF could be attributed to more susceptibility of NDF to water stress. Therefore, more lignin could have been made at drought stress conditions. The higher ADF and NDF values in the dry year (2015-2016) compared with the wet year (2016-2017) of this study could be explained by lower precipitation (86.4 mm) in 2015-2016 compared with that (572 mm) in 2016-2017 and higher mean temperature (17.2 ºC) in reproductive period (March) of triticale life cycle in the first year than that (14.1 ºC) the second year (Fig. 1 & Table 4). These results further confirmed the findings of Maleki farahani and Chaichi (2013), who showed that the ADF and NDF increased in dry and warm conditions. In addition, higher fiber content and low DMD under elevated temperature and dry season reported by other authors (Safari et al., 2011; Seligman and Sinclair, 1995).

Increasing of N concentration and/or crude protein (CP) with improving severity of water stress in the current study (Fig. 4) is in line with findings of Barati et al., (2015), Ozturk and Aydin (2004) and Haberle et al. (2008). The increment of CP due to drought stress was different in N treatments, the highest increment occurred in Bio + N_{75} treatment, followed by Bio treatment compared with N_{150} treatment (Fig. 4). The better performance of biological N fertilizers for production of CP of forage triticale under water stress condition can be attributed to higher N uptake from soil during drought stress conditions (data not shown). Field experiments that were performed with *Azospirillum*-inoculated crops in different areas have significantly shown increased water and mineral uptake (Ozturk et al., 2003; Creus et al., 2004; Dobbelaere et al., 2001). It was reported that the adequate P uptake in biological fertilizer applications can enhance crop water stress resistance through different mechanisms, such as improving root growth and development, which in turn extends water and nutrients (including N) absorption (Wu et al., 2013). Furthermore, increasing root hydraulic conductance in biofertilizer treatments was reported by Singh and Sale (2000). In contrast, the lower CP in N_{150} treatment compared with the integrated N fertilizer under drought stress conditions in this study (Fig. 4) could be attributed to increasing severity of water stress in N_{150} than those in the other N treatments and consequently reducing N uptake by triticale crops. Ercoli et al. (2008) reported that the plants which received high chemical N fertilizer were more sensitive to drought than the unfertilized ones. They showed that accumulation of N greatly reduced when water stress occurred as well, especially in high N fertilizer application.

The significant decreasing and increasing of forage ash content in chemical N fertilizer alone and integrated N fertilizer applications, respectively, as a consequence of improving water stress in the current study (Table 5) further confirmed the Maleki Farahani and Chaichi (2013) findings. Jorgensen et al. (1999) reported similar results about ash content of barley grain. They showed that the ash content was negatively influenced by higher N availability.

Table 5. Effects of irrigation regimes × N fertilizer sources on acid detergent fiber (ADF) and ash in 2015-2016 and 2016-2017.

Year	Irrigation regime	N fertilizer source N_0 §	N_{150}	Bio	Bio + N_{75}	Mean
			Acid detergent fiber (ADF) (g kg^{-1})			
2015-2016	IR_N £	38.46^{b}	38.73^{ab}	37.35^{bc}	38.01^{bc}	38.14
	IR_{MD}	39.78^{ab}	42.18^{a}	34.74^{c}	34.52^{c}	37.81
	IR_0	–	–	–	–	
	Mean	39.12	40.46	36.05	36.27	
	$LSD_{(0.05)}$ = 3.65					
2016-2017	IR_N	36.01^{cde}	36.11^{cde}	33.43^{ef}	34.21^{def}	34.94
	IR_{MD}	37.03^{cd}	38.42^{bc}	32.12^{fg}	31.89^{fg}	34.87
	IR_0	41.56^{ab}	44.96^{a}	28.98^{gh}	28.00^{h}	35.88
	Mean	38.20	39.83	31.51	31.37	
	$LSD_{(0.05)}$ = 3.56					
			Ash (%)			Mean
2015-2016	IR_N	5.47^{b}	5.32^{b}	5.55^{b}	5.86^{b}	5.55
	IR_{MD}	5.52^{b}	4.43^{c}	5.47^{b}	6.83^{a}	5.56
	IR_0	–	–	–	–	
	Mean	5.50	4.88	5.51	6.35	
	$LSD_{(0.05)}$ = 0.70					
2016-2017	IR_N	6.18^{c}	5.68^{cd}	5.74^{cd}	6.42^{bc}	6.01
	IR_{MD}	6.19^{bc}	5.24^{de}	5.70^{cd}	7.07^{ab}	6.05
	IR_0	6.47^{bc}	4.79^{e}	5.61^{cde}	7.88^{a}	6.19
	Mean	6.28	5.24	5.68	7.12	
	$LSD_{(0.05)}$ = 0.90					

Means in each interaction followed by the same letters are not significantly different at 5% probability level according to Fisher's LSD test. £ IR_N: normal irrigation, IR_{MD}: mild deficit irrigation, IR_0: Rainfed.
§ N_0: no N fertilizer (control), N_{150}: sole chemical N fertilizer [150 kg N ha^{-1}], Bio: sole biological fertilizer (seed inoculated with *Azospirillum brasilense*), Bio + N_{75}: combined fertilizer (50% chemical N fertilizer [75 kg N ha^{-1}] + seed inoculated by *Azospirillum brasilense*).

CONCLUSIONS

According to the results of this study it was found that cutting off irrigation after anthesis stage decreased forage quality indexes of triticale such as L/S ratio, DMD and increased NDF and ADF when conventional chemical N fertilizer was applied. However, the adverse effect of water stress was diminished with applying of biofertilizer alone or in combination with the chemical N fertilizer, because these N fertilizer sources increased L/S ratio and decreased ADF in drought stress conditions and DMD was not drastically affected by late season water stress as well. Indeed, application of this irrigation strategy in combination with integrated chemical N fertilizer and biofertilizer supply increased forage triticale quality without any drastic decrease in dry matter. Furthermore, cutting off irrigation after anthesis stage for triticale not only reduces irrigation water consumption but also can provide irrigation water for a larger number of the farmers in arid zones.

ACKNOWLEDGMENTS

The authors are grateful to Shiraz University for providing the financial support for the project.

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تحقیقات کشاورزی ایران (۱۳۹۹) ۳۹(۲) ۶۹-۸۰

مقاله علمی- پژوهشی

کیفیت علوفه‌ای تریتیکاله تحت تاثیر تنش آبی و کود زیستی نیتروژن در شرایط اقلیمی خشک

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اطلاعات مقاله

تاریخچه مقاله:

تاریخ دریافت: ۱۳۹۹/۵/۲۱

تاریخ پذیرش: ۱۳۹۹/۱۰/۱۵

تاریخ دسترسی: ۱۳۹۹/۱۱/۱۱

واژه‌های کلیدی:

آزوسپیریلوم

پروتئین خام

قابلیت گوارش ماده خشک

آبیاری

چکیده- فهم برهمکنش آب و قابلیت دسترسی به کود نیتروژن موضوع مهمی برای ثبات تولید علوفه در مناطق خشک است. این مطالعه، برهمکنش منابع نیتروژن و تنش آبی بر مهمترین ویژگی-هایی از گیاه تریتیکاله (*Triticosecale* Wittmack×) که در کیفیت علوفه‌ای آن نقش دارند را در منطقه‌ای با شرایط اقلیمی خشک در ایران (داراب) در سال‌های زراعی ۲۰۱۶-۲۰۱۵ و ۲۰۱۷-۲۰۱۶ مورد بررسی قرار داده است. رژیم‌های آبیاری در سال نخست شامل دو سطح: یکی آبیاری مطلوب و دیگری قطع آبیاری پس از مرحله‌ی گلدهی (تنش آبی) بودند. در سال دوم، شرایط دیم نیز به عنوان تیمار سوم به آزمایش اضافه شد. چهار منبع نیتروژن شامل ۱- باکتری آزوسپیریلوم به‌عنوان تیمار زیستی ۲- استفاده از باکتری آزوسپیریلوم + ۷۵ کیلوگرم نیتروژن در هکتار به صورت اوره به‌عنوان تیمار تلفیقی ۳- کاربرد ۱۵۰ کیلوگرم نیتروژن در هکتار به صورت اوره به‌عنوان تیمار شیمیایی نیتروژن و ۴- عدم کاربرد نیتروژن به‌عنوان تیمار شاهد بودند. تنش آبی سبب کاهش نسبت برگ به ساقه (L\S)، وزن خشک اندام هوایی (DM) و گوارش پذیری ماده‌ی خشک (DMD) و افزایش مقدار پروتئین خام (CP)، فیبرهای نامحلول در شوینده‌های خنثی (NDF) و مقدار خاکستر شد. اما، اثر تنش آبی بر مقدار خاکستر، گوارش پذیری ماده‌ی خشک، فیبرهای نا محلول در شوینده‌های اسیدی، نسبت برگ به ساقه (در سال زراعی ۲۰۱۶-۲۰۱۵) و مقدار پروتئین خام، وابسته به کاربرد منبع نیتروژن بود (برهمکنش معنی‌دار کود نیتروژن × رژیم آبیاری). بالاترین مقدار پروتئین خام، مقدار گوارش پذیری ماده‌ی خشک، مقدار خاکستر و نسبت برگ به ساقه (در سال زراعی ۲۰۱۶-۲۰۱۵) و کمترین مقدار فیبر نامحلول در شوینده‌های اسیدی با استفاده از کود تلفیقی در مقایسه با سایر منابع نیتروژن در شرایط تنش آبی بدست آمد. با توجه به نتایج این مطالعه می توان پیشنهاد نمود که از تلفیق کود زیستی (باکتری آزوسپیریلوم) و کود شیمیایی نیتروژن (۷۵ کیلوگرم در هکتار) به‌طور موفقیت آمیزی می‌توان در جهت افزایش کیفیت علوفه تریتیکاله در شرایط تنش آبی در سامانه‌های پایدار کشاورزی مناطق خشک ایران استفاده نمود.