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Original Article

Effect of drought stress on morpho-physiological characteristics, nutritive value, and water-use efficiency of sorghum [*Sorghum bicolor* (L.) Moench] varieties under various irrigation systems

Efeito do estresse hídrico sobre características morfofisiológicas, valor nutritivo e eficiência no uso da água de variedades de sorgo [*Sorghum bicolor* (L.) Moench] sob diferentes sistemas de irrigação

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Abstract

Addressing water scarcity and the need for high-quality forage in arid regions necessitates the development of efficient irrigation techniques. This study assesses the impact of various irrigation methods on the performance and irrigation water-use efficiency (IWUE) of sorghum cultivars under water-deficit conditions in a semi-arid region of Iran during the 2019 and 2020 cropping seasons. Three irrigation methods—variable alternate furrow irrigation (AFI), fixed alternate furrow irrigation (FFI), and conventional furrow irrigation (CFI)—were evaluated alongside three levels of drought stress (severe stress: I_{50}, moderate stress: I_{75}, and full irrigation: I_{100}) and two sorghum cultivars. The results indicated that increasing drought stress, as well as the transition from CFI to AFI and FFI, led to reductions in metabolizable energy yield (MEY), plant height, cellulose, hemicellulose, and lignin. Conversely, there were increases in leaf-to-stem ratio, digestible organic matter, metabolizable energy content, crude protein content, and IWUE for metabolizable energy production ($IWUE_{ME}$). The highest MEY (211.68 GJ ha^{-1}) was recorded under CFI×I_{100}, albeit at the expense of maximum water consumption (7261 m^3 ha^{-1}). Meanwhile, the AFI×I_{50} and FFI×I_{50} treatments exhibited the highest $IWUE_{ME}$ (44.46 MJ m^{-3}) and metabolizable energy content (8.736 MJ kg^{-1}), respectively, while conserving over 60% of water. Hybrid Speedfeed outperformed in forage yield and $IWUE_{ME}$, while cultivar Pegah excelled in forage quality. Transitioning from CFI to AFI or FFI resulted in decreased forage yield but improved forage quality and $IWUE_{ME}$. Principal component analysis revealed that leaf-to-stem ratio and plant height serve as effective indicators for assessing the nutritive value and forage yield of sorghum, respectively. Considering the overall results, cultivating the hybrid Speedfeed under AFI×I_{75} conditions is recommended for optimal water utilization, achieving satisfactory forage yield and quality, and enhancing IWUE.

Keywords: cell wall, deficit irrigation, metabolizable energy, partial root-zone drying, protein

Resumo

Abordar a escassez de água e a necessidade de forragem de alta qualidade em regiões áridas exige o desenvolvimento de técnicas eficientes de irrigação. Este estudo avalia o impacto de vários métodos de irrigação no desempenho e na eficiência do uso da água de irrigação (IWUE) de cultivares de sorgo sob condições de déficit hídrico em uma região semiárida do Irã durante as temporadas de cultivo de 2019 e 2020. Três métodos de irrigação – irrigação alternada variável por sulcos (AFI), irrigação alternada fixa por sulcos (FFI) e irrigação convencional por sulcos (CFI) – foram avaliados juntamente com três níveis de estresse hídrico (estresse severo: I_{50}, estresse moderado: I_{75} e irrigação total: I_{100}) e dois cultivares de sorgo. Os resultados indicaram que o aumento do estresse hídrico, bem como a transição de CFI para AFI e FFI, levaram a reduções no rendimento de energia metabolizável (MEY), na altura da planta, na celulose, na hemicelulose e na lignina. Por outro lado, houve aumentos na relação folha-caule, na matéria orgânica digestível, no conteúdo de energia metabolizável, no conteúdo de proteína bruta e na IWUE para produção de energia metabolizável ($IWUE_{ME}$). O maior MEY (211,68 GJ ha^{-1}) foi registrado sob CFI×I_{100}, embora com o maior consumo de água (7261 m^3 ha^{-1}). Enquanto isso, os tratamentos AFI×I_{50} e FFI×I_{50} apresentaram a maior $IWUE_{ME}$ (44,46 MJ m^{-3}) e o maior conteúdo de energia metabolizável (8,736 MJ kg^{-1}), respectivamente, conservando mais de 60% de água. O híbrido Speedfeed superou em rendimento de forragem e $IWUE_{ME}$, enquanto a cultivar Pegah destacou-se na qualidade da forragem. A transição de CFI para AFI ou FFI resultou em menor rendimento de forragem, mas melhorou a qualidade da forragem e a $IWUE_{ME}$. A análise de componentes principais revelou

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que a relação folha-caule e a altura da planta servem como indicadores eficazes para avaliar o valor nutritivo e o rendimento de forragem do sorgo, respectivamente. Considerando os resultados gerais, recomenda-se o cultivo do híbrido Speedfeed sob as condições de AFI×I_{75} para a utilização ótima da água, alcançando rendimento e qualidade de forragem satisfatórios, e melhorando a IWUE.

Palavras-chave: parede celular, irrigação deficitária, energia metabolizável, secagem parcial da zona radicular, proteína

1. Introduction

Climate change and limited water resources have put food security in semi-arid and arid nations at risk recently (Hosseini et al., 2022; Ghalkhani et al., 2023). Thus, in these areas, conserving irrigation water requires appropriate procedures (Aghajani et al., 2023; Pourali et al., 2023). One of the acceptable methods to lower the quantity of water used is to use partial root-zone drying (PRD) techniques and regulated deficit irrigation (RDI) systems (Alzoheiry, 2024). The PRD technique, known as alternate furrow irrigation, involves watering half of the plant's root system while keeping the other half devoid of water (Golzardi et al., 2017; Aghajani et al., 2023). To increase irrigation water-use efficiency (IWUE) while maintaining an acceptable yield, crops are subjected to controlled drought stress via RDI systems and PRD techniques (Iqbal et al., 2020; Shirinpour et al., 2021). Comparative to conventional furrow irrigation (CFI), employing PRD methods under water deficit conditions has been shown to enhance yield stability, increase IWUE, reduce soil surface evaporation, foster root growth and development, and mitigate the plant's susceptibility to drought stress (Stoll et al., 2000; Yang et al., 2010; Jia et al., 2014). Many researchers, including Kang et al. (2000), Golzardi et al. (2017), Iqbal et al. (2020), and Baghdadi et al. (2023), have demonstrated that the PRD strategy positively impacts IWUE, as well as root growth and development.

In water-scarce regions, cultivating drought-tolerant crops like sorghum is preferred over water-intensive crops like maize and clover due to their superior ability to thrive with limited water (Bakhtiyari et al., 2020; Khalilian et al., 2022). Moreover, high biomass output and high-quality yield are essential when selecting forage species in areas with limited irrigation (Pourali et al., 2023). Sorghum's resilience to heat and drought stress, efficient water usage, and high biomass output make it suitable for arid environments (Khalilian et al., 2022; Sürmen and Kara, 2022). Several studies, including those by Jahanzad et al. (2013), Kaplan et al. (2019), Farhadi et al. (2022), and Ghalkhani et al. (2023), have demonstrated that drought stress can enhance desirable characteristics of sorghum forage. Sorghum holds significant promise as a sustainable forage crop, particularly in regions prioritizing both forage quantity and quality, especially those with limited irrigation resources (Bhattarai et al., 2020).

Choosing the proper sorghum cultivars improves forage yield, feed value, and IWUE (Khalilian et al., 2022). Sorghum cultivars exhibit varying levels of drought tolerance, photosynthetic rates, and leaf-to-stem ratios, which influence their forage production potential and IWUE (Jahanzad et al., 2013). Cultivars with higher drought tolerance or faster growth rates can enhance IWUE by maximizing production per unit of water (Golzardi et al., 2017). Moreover, varieties with a higher leaf-to-stem ratio receive more light, have a faster photosynthesis rate, and produce and grade better forage (Jia et al., 2022; Ghalkhani et al., 2023). Given the escalating demand for feed, the scarcity of irrigation water in arid regions and the importance of sorghum in livestock, exploring diverse irrigation techniques' impacts on sorghum production is imperative. Studying how water deficits and PRD irrigation methods influence forage production and IWUE in sorghum cultivars aids in devising strategies to alleviate drought stress and enhance sustained forage output. Consequently, this research examined sorghum cultivars' responses to varied irrigation methods across varying drought stress levels, prioritizing forage yield and quality, and IWUE.

2. Materials and methods

2.1. Experimental site and details

The study was conducted at the Seed and Plant Improvement Institute in Karaj, Iran (35°47'N, 50°55'E, 1321 m elevation) during the 2019 and 2020 cropping seasons, under semi-arid climatic conditions. Detailed information on air temperature, evaporation, and precipitation during the two cropping seasons at the experimental site is documented in Table 1. Additionally, Table 2 provides an overview of the soil characteristics at the experimental site. A factorial split-plot design with three replications was employed. Main plots were designated for various irrigation methods and regimes, while sub-factors included the open-pollinated cultivar Pegah and hybrid Speedfeed. Two PRD irrigation methods, AFI and FFI, were compared with CFI. Three irrigation regimes were implemented: severe drought stress (I_{50}), moderate drought stress (I_{75}), and full irrigation (I_{100}). These regimes aimed to supply 50, 75, and 100% of the soil moisture deficit, respectively. Seedbed preparation included plowing, harrowing, and leveling, with soil being enriched with urea (100 kg ha^{-1}) and diammonium phosphate (250 kg ha^{-1}) during initial planting. Sub-plots consisted of four rows, each 5 m long, with a within-row planting distance of 8 cm and ridge-to-ridge spacing of 60 cm. Sowing occurred on June 1st, 2021, and June 2nd, 2022, with additional urea administered through fertigation at the 4-leaf stage.

2.2. Irrigation

The required amount of irrigation water was determined by measuring the soil moisture content at the root depth using a time-domain reflectometry (TDR) device. This measurement aimed to accurately determine the specific water quantity needed for the full irrigation regime by calculating the difference between the soil moisture content at the field capacity (FC) point (θ_{FC}) and the soil moisture content before each irrigation cycle (θ_i). Equation 1 was utilized for this estimation (Afshar et al., 2014):

$$V_w = \left(\theta_{FC} - \theta_i\right) \times D \times A \quad (1)$$

Irrigation was scheduled to be applied every seven days. For water transportation to the furrows, 5-cm polyethylene pipes were utilized. Within each plot, four taps were installed on the pipes, allowing water access at 60-cm intervals along the furrows. The distribution of water varied depending on the irrigation method employed. In the CFI method, water was uniformly distributed across all furrows. However, in the AFI and FFI methods, water was selectively administered to one of every two adjacent furrows. In the AFI method, the furrows were alternated during subsequent irrigation cycles. On the other hand, the FFI method consistently targeted only the first and third furrows throughout the season. A distance of 3 m was maintained between the main plots, and a distance of 4 m was maintained between the blocks to avoid water leakage into adjacent plots. The total volumes of irrigation water used in different irrigation methods under three levels of drought stress during the two growing seasons are presented in Table 3.

2.3. Samplings and measurements

The dry matter yield (DMY) was assessed by harvesting the central two rows of each plot after the vegetative growth phase. The forage was collected in two separate cuts, and the total DMY was calculated by summing the production from both harvests. Van Soest's method (Van Soest et al., 1991) was employed to measure the ADF (cellulose and lignin) and NDF (cellulose, hemicellulose, and lignin).

Table 1. Monthly average of air temperature, evaporation, and precipitation during the two growing seasons at the experimental site.

Year	Month	T_{mean} (°C)	T_{max} (°C)	T_{min} (°C)	Evaporation (mm)	Precipitation (mm)
2019	June	27.6	35.0	19.1	297	11
	July	29.1	37.5	20.5	353	1
	August	26.7	34.8	18.8	300	8
	September	22.2	30.8	14.3	208	0
	October	16.8	23.5	11.2	131	76
2020	June	27.8	35.6	19.4	306	2
	July	29.6	38.3	20.2	372	20
	August	27.3	36.2	18.3	312	3
	September	22.8	31.5	14.3	222	0
	October	17.1	24.2	11.0	135	42

T_{mean} – mean temperature, T_{min} – minimum temperature, T_{max} – maximum temperature.

Table 2. Physical and chemical properties of the soil at the experimental site during the two growing seasons.

Year	Texture	pH	N (%)	P (mg kg^{-1})	K (mg kg^{-1})	OM† (%)	EC (dS m^{-1})	FC (%)	PWP (%)
2019	Clay loam	7.2	0.06	12.5	255	0.57	2.1	33	11
2020	Clay loam	7.1	0.07	12.7	249	0.58	2.0	32	10

OM – organic matter, EC – electrical conductivity, FC – field capacity, PWP – permanent wilting point.

Table 3. Total volume of irrigation water used (m^3 ha^{-1}) in different irrigation methods under three levels of drought stress during the two growing seasons.

Drought stress	2019			2020		
	CFI	FFI	AFI	CFI	FFI	AFI
I_{100}	7215	5206	5595	7307	5310	5749
I_{75}	5411	3905	4196	5480	3983	4312
I_{50}	3608	2603	2798	3654	2655	2875

CFI – conventional furrow irrigation, FFI – fixed alternate furrow irrigation, AFI – variable alternate furrow irrigation, I_{100} – full irrigation, I_{75} – moderate drought stress, I_{50} – severe drought stress.

The hemicellulose (HCEL) content was determined as the difference between the NDF and ADF values. The ash content of the samples was assessed by subjecting them to combustion in a furnace at a temperature of 600 °C for a duration of 8 h (AOAC, 2000). The Kjeldahl method was employed to determine the crude protein content (CPC), utilizing Equation 2 (Kjeldahl, 1883):

$$CPC = Total\ Nitrogen \times 6.25 \tag{2}$$

Metabolizable energy (ME) and digestible organic matter (DOM) were measured using the *in vitro* method, utilizing Equations 3 and 4 (Blümmel et al., 1997; Menke et al., 1979). Additionally, Equation 5 was utilized to determine the relative feed value (RFV) (Lithourgidis et al., 2006):

$$ME = (0.136 \times GPD) + (0.057 \times CPC) + 2.2 \tag{3}$$

$$DOM = (0.889 \times GPD) + (0.45 \times CPC) + (0.0651 \times Ash) + 14.88 \tag{4}$$

$$RFV = DDM \times DMI \times 0.775 \tag{5}$$

In Equations 3 and 4, *GPD* represents the net gas production over a 24-hour period (milliliters per 200 mg of DM). In Eq. 5, DDM represents DM digestibility (%), and DMI signifies DM intake (% of livestock body weight). The yields of digestible organic matter (DOMY), crude protein (CPY), and metabolizable energy (MEY) were determined by multiplying their respective contents by the DMY. The IWUE in ME production ($IWUE_{ME}$) was calculated using Equation 6 (Baghdadi et al., 2023):

$$IWUE_{ME} = MEY \ / \ IWU \tag{6}$$

In Eq. 6, MEY represents the ME yield (MJ ha^{-1}), and IWU denotes the overall amount of irrigation water utilized throughout the growing season (m^3 ha^{-1}).

2.4. Statistical analysis

Prior to statistical analysis, data distribution was examined using the Shapiro-Wilk test, confirming their normality. Subsequently, given the results of Bartlett's test and the homogeneity of experimental errors over the two years of the study, a combined analysis of variance was performed. The data was subjected to a combined analysis of variance, treating the year as a random effect. Statistical analyses were conducted using the general linear model (GLM) procedures of SAS 9.1, and mean comparisons were executed by employing the LSD method (p≤0.05). Due to the non-significant interaction between the year and treatments, the two-year average of traits was reported. Principal component analysis (PCA) was performed using Minitab 21 software to investigate the interrelationships between the experimental factors and the measured characteristics. The analysis employed the correlation matrix of both quantitative and qualitative traits.

3. Results

3.1. Dry matter yield

The maximum DMY was achieved under CFI×I_{100}, while the minimum was in FFI×I_{50}. Across all irrigation methods, an increase in drought stress severity led to a decrease in DMY, with CFI exhibiting the highest rate of decrease and AFI showing the lowest. Compared to I_{100}, the I_{75} and I_{50} regimes under CFI resulted in DMY decreases of 17.3% and 35.0%, respectively. Similarly, under AFI, these regimes led to decreases of 15.6% and 28.3%, respectively (Table 4). While no significant difference in DMY was observed between the two cultivars under CFI, Speedfeed consistently outperformed Pegah in other irrigation methods (Table 5). Additionally, Speedfeed consistently outperformed Pegah across all irrigation regimes, increasing its superiority with drought stress intensity (Table 6).

3.2. Digestible organic matter

The highest DOM and the lowest DOMY were recorded under FFI×I_{50}, whereas the lowest DOM and maximum DOMY were obtained under CFI×I_{100}. As the water deficit increased, DOMY decreased, while DOM increased across all irrigation methods (Tables 4 and 7). Speedfeed had higher DOMY than Pegah in all irrigation methods, with significant superiority observed only under FFI (Table 5). The superiority of Speedfeed over Pegah increased with the severity of drought stress (Table 6).

3.3. Crude protein

Increasing drought stress severity in all irrigation methods led to a notable decrease in CPY and a significant improvement in CPC. Pegah consistently exhibited higher CPC and CPY than Speedfeed across all irrigation methods and regimes (Figure 1). The highest CPY was achieved by the Pegah cultivar under CFI×I_{100}, while the lowest was in Speedfeed under FFI×I_{50} (Figure 1a). Speedfeed under CFI×I_{100} recorded the lowest CPC, whereas the Pegah under FFI×I_{50} exhibited the highest CPC (Figure 1b). The most significant decrease in CPY due to increased drought stress intensity was observed in Pegah under CFI, while the highest increase in CPC was observed in Pegah under FFI (Figure 1).

3.4. Metabolizable energy

As drought stress severity increased, ME increased while MEY decreased. The highest ME and the lowest MEY were obtained under FFI×I_{50}, whereas the highest MEY and the lowest ME were obtained under CFI×I_{100} (Tables 4 and 7). Speedfeed consistently had higher MEY than Pegah across all irrigation methods, with significant superiority observed only under FFI. The superiority of Speedfeed over Pegah under the CFI, AFI, and FFI methods was 3.5%, 5.8%, and 19.8%, respectively (Table 5). Similarly, Speedfeed had a higher MEY than Pegah in all irrigation regimes. The superiority of Speedfeed over Pegah under the I_{100}, I_{75}, and I_{50} regimes was 3.5%, 11.2%, and 13.6%, respectively (Table 6).

Table 4. Effects of irrigation method × drought stress on forage yield and leaf-to-stem ratio.

Irrigation method (I)	Drought stress (D)	Dry matter yield (t ha^{-1})	Digestible organic matter yield (t ha^{-1})	Metabolizable energy yield (GJ ha^{-1})	Leaf-to-stem ratio
CFI	I_{100}	25.76	14.28	211.68	0.578
	I_{75}	21.31	11.92	176.60	0.604
	I_{50}	16.74	9.67	143.30	0.637
	$LSD_{0.05}$	0.94	0.45	6.67	0.017
FFI	I_{100}	18.58	10.73	158.91	0.645
	I_{75}	15.41	8.95	132.60	0.658
	I_{50}	12.08	7.10	105.04	0.675
	$LSD_{0.05}$	0.85	0.44	6.42	0.016
AFI	I_{100}	20.47	11.75	174.11	0.624
	I_{75}	17.28	9.97	147.57	0.641
	I_{50}	14.67	8.52	126.11	0.662
	$LSD_{0.05}$	0.83	0.49	7.27	0.010
$LSD_{0.05}$ (I×D)		0.53	0.25	3.77	0.004

CFI – conventional furrow irrigation, FFI – fixed alternate furrow irrigation, AFI – variable alternate furrow irrigation, I_{100} – full irrigation, I_{75} – moderate drought stress, I_{50} – severe drought stress.

Table 5. Effects of irrigation method × cultivar on yield and nutritive value of sorghum forage.

Irrigation method (I)	Cultivar (C)	DMY	DOMY	MEY	LSR	NDF	HCEL
		(t ha^{-1})		(GJ ha^{-1})		(g kg^{-1})	
CFI	Speedfeed	22.25	12.16	180.23	0.587	592.7	191.3
	Pegah	20.29	11.76	174.16	0.626	578.5	206.4
	$LSD_{0.05}$	ns	ns	ns	0.037	10.3	12.2
FFI	Speedfeed	17.16	9.72	144.11	0.631	562.6	178.4
	Pegah	13.56	8.13	120.25	0.687	547.7	191.9
	$LSD_{0.05}$	3.25	1.24	20.17	0.045	3.9	1.2
AFI	Speedfeed	18.45	10.35	153.47	0.611	579.4	190.1
	Pegah	16.49	9.81	145.06	0.674	558.5	197.1
	$LSD_{0.05}$	1.44	ns	ns	0.059	4.2	ns
$LSD_{0.05}$ (I×C)		0.68	0.31	4.66	0.008	2.0	2.4

CFI – conventional furrow irrigation, FFI – fixed alternate furrow irrigation, AFI – variable alternate furrow irrigation, DMY – dry matter yield, DOMY – digestible organic matter yield, MEY – metabolizable energy yield, LSR – leaf-to-stem ratio, NDF – cellulose, hemicellulose and lignin, HCEL – hemicellulose, CPC – crude protein content.

3.5. IWUE for energy production

With increasing drought stress intensity, $IWUE_{ME}$ increased, with AFI showing the highest increase. The highest $IWUE_{ME}$ was observed in Speedfeed under AFI×I_{50}, whereas the lowest $IWUE_{ME}$ was noted in Pegah under FFI×I_{100}. Both cultivars had low $IWUE_{ME}$ under CFI×I_{100} (Figure 2a). Speedfeed consistently exhibited significantly higher $IWUE_{ME}$ than Pegah across all combinations of irrigation methods and regimes. Both cultivars showed an increase in $IWUE_{ME}$ with escalating drought stress, but the rate of increase was notably higher in Speedfeed. While both cultivars had similar $IWUE_{ME}$ under CFI×I_{100}, a transition to PRD methods combined with intensified drought stress severity resulted in significantly higher $IWUE_{ME}$ in Speedfeed than Pegah (Figure 2a).

3.6. Morphological characteristics

The maximum and minimum leaf-to-stem ratio (LSR) were recorded under FFI×I_{50} and CFI×I_{100}, respectively. Increasing drought stress increased LSR across all

Table 6. Effects of drought stress × cultivar on forage yield of sorghum.

Drought stress (D)	Cultivar (C)	Dry matter yield	Digestible organic matter yield	Metabolizable energy yield
		(t ha^{-1})		(GJ ha^{-1})
I_{100}	Speedfeed	22.57	12.46	184.71
	Pegah	20.64	12.05	178.42
	$LSD_{0.05}$	1.14	ns	ns
I_{75}	Speedfeed	19.48	10.82	160.34
	Pegah	16.52	9.74	144.17
	$LSD_{0.05}$	0.93	0.55	8.12
I_{50}	Speedfeed	15.81	8.95	132.75
	Pegah	13.18	7.90	116.88
	$LSD_{0.05}$	0.60	0.34	5.01
$LSD_{0.05}$ (D×C)		0.28	0.17	2.58

I_{100} – full irrigation, I_{75} – moderate drought stress, I_{50} – severe drought stress.

Table 7. Effects of irrigation method × drought stress on nutritive value of sorghum forage.

Irrigation method (I)	Drought stress (D)	ADF	NDF	HCEL	Ash	DOM	ME	RFV
				(g kg^{-1})			(MJ kg^{-1})	(%)
CFI	I_{100}	394.9	604.1	209.2	91.0	555.0	8.225	89.60
	I_{75}	391.0	588.9	197.9	92.2	560.4	8.303	92.35
	I_{50}	374.4	563.8	189.4	95.0	579.0	8.581	98.63
	$LSD_{0.05}$	1.7	8.2	6.6	0.6	4.3	0.066	1.27
FFI	I_{100}	374.5	567.0	192.5	94.7	578.5	8.571	98.05
	I_{75}	370.1	558.2	188.1	95.8	583.4	8.638	100.18
	I_{50}	365.5	540.3	174.9	96.5	590.3	8.736	104.11
	$LSD_{0.05}$	2.9	9.1	11.3	1.2	0.7	0.010	1.13
AFI	I_{100}	378.4	579.0	200.6	94.1	574.8	8.516	95.56
	I_{75}	375.4	568.8	193.4	94.7	577.9	8.557	97.65
	I_{50}	372.0	559.0	187.0	95.2	581.8	8.610	99.77
	$LSD_{0.05}$	3.2	12.0	11.9	0.5	3.3	0.047	2.09
$LSD_{0.05}$ (I×D)		1.8	3.5	3.0	0.5	1.9	0.029	0.69

CFI – conventional furrow irrigation, FFI – fixed alternate furrow irrigation, AFI – variable alternate furrow irrigation, I_{100} – full irrigation, I_{75} – moderate drought stress, I_{50} – severe drought stress, ADF – cellulose and lignin, NDF – cellulose, hemicellulose, and lignin, HCEL – hemicellulose, DOM – digestible organic matter, ME – metabolizable energy content, RFV – relative feed value.

irrigation methods, notably in CFI (Table 4). Speedfeed consistently had significantly lower LSR than the Pegah under all irrigation methods (Table 5). The maximum plant height (PLH) was observed in Speedfeed under CFI×I_{100}, while the minimum was in Pegah under FFI×I_{50} (Figure 2b). Both cultivars showed decreased PLH with increasing drought stress severity, but Pegah was more sensitive, especially in FFI and AFI. Speedfeed consistently exhibited higher PLH than Pegah across all irrigation methods and regimes, with the slightest difference under CFI (Figure 2b).

3.7. Cell wall, ash, and relative feed value

Increasing drought stress severity decreased fiber concentration and increased ash content, notably under CFI. The RFV increased with higher water deficit intensity, most notably in CFI. The highest and lowest RFV were recorded under FFI×I_{50} and CFI×I_{100}, respectively (Table 7). FFI×I_{50} had the lowest ADF, NDF, and HCEL and the highest ash content, while CFI×I_{100} had the highest ADF, NDF, and HCEL and the lowest ash content (Table 7). Speedfeed consistently had higher NDF than Pegah across all methods, while Pegah had higher HCEL content (Table 5).

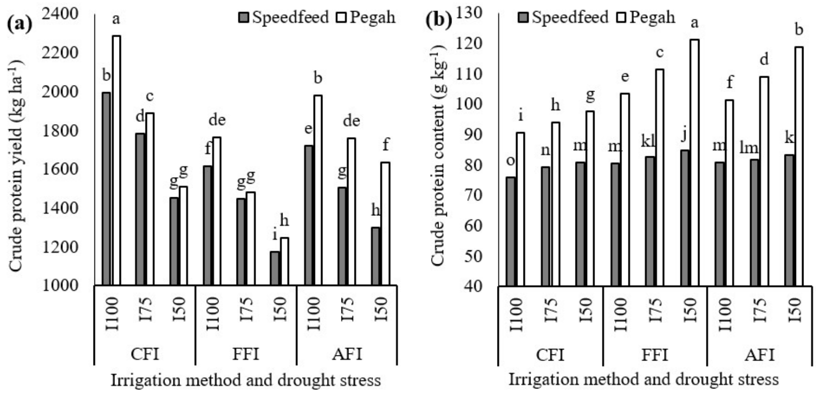

Figure 1. Effects of irrigation method × drought stress × cultivar interaction on crude protein yield (a), and crude protein content (b); Explanation as in Table 4.

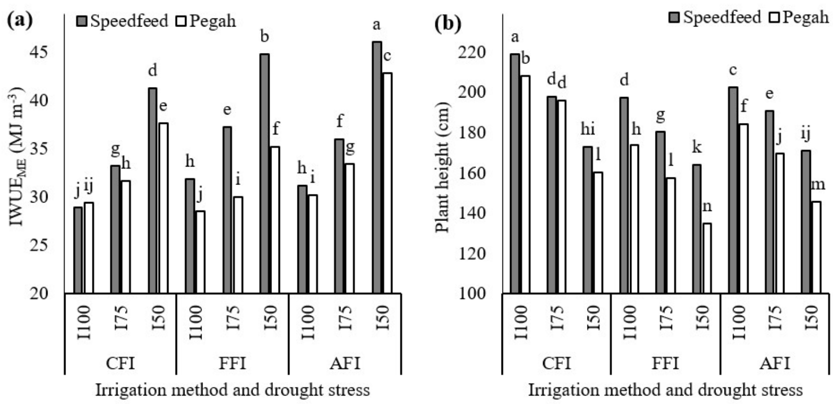

Figure 2. Effects of irrigation method × drought stress × cultivar interaction on $IWUE_{ME}$ (a), and plant height (b); Explanation as in Table 4.

3.8. Principal component analysis

The PCA was used to analyze multiple traits (Figure 3). Eigenvalues and proportion of total variance represented by first five principal components are presented in Table 8. The first principal component showed negative correlations with RFV, LSR, ash, ME, DOM, and CPC but positive correlations with DMY, PLH, MEY, NDF, DOMY, and ADF. The second principal component had a negative correlation with HCEL and a positive correlation with $IWUE_{ME}$ (Figure 3b). The NDF content exhibited the strongest positive correlations with PLH, forage yield, and energy yield, whereas it demonstrated the strongest negative correlation with RFV and LSR. The RFV positively correlated with LSR, ME, DOM, ash, and CPC. $IWUE_{ME}$ demonstrated the highest negative correlation with HCEL and CPY (Figure 3b). The PLH exhibited the highest correlation with DMY, MEY, DOMY, and NDF. In contrast, LSR positively correlated with RFV, ME, ash, DOM, and CPC. Therefore, plant height indicated quantitative yield, while leaf-to-stem ratio indicated nutritional value (Figure 3b). CFI resulted in the highest forage yield and energy production per unit area, while FFI had the highest energy content and nutritional value (Figure 3a). Transitioning from CFI to AFI and FFI improved forage quality but decreased yield. AFI had a higher yield than FFI and better quality than CFI. Increased drought stress improved forage quality and IWUE at the expense of yield. Cultivar Pegah had superior forage quality, while hybrid Speedfeed excelled in yield and IWUE. Cultivar Pegah recorded the highest forage quality under the FFI × I_{50} treatment, whereas the hybrid Speedfeed achieved the highest forage yield under the CFI × I_{100}. Maximum $IWUE_{ME}$ was observed in the hybrid Speedfeed under severe drought stress and alternate furrow irrigation methods (Figure 3a).

4. Discussion

4.1. Forage yield

The study reaffirms drought stress's adverse effects on sorghum forage yield, consistent with prior research (Pourali et al., 2023; Baghdadi et al., 2023). Drought impedes plant growth and physiological processes, lowering biomass production (Farhadi et al., 2022; Aghajani et al.,

Table 8. Principal component analysis of quantitative and qualitative traits of sorghum affected by experimental treatments.

Variable	PC_1	PC_2	PC_3	PC_4	PC_5
DMY	0.290	-0.141	0.084	-0.208	0.071
DOMY	0.278	-0.198	0.125	-0.299	-0.009
CPY	0.181	-0.417	0.282	-0.291	0.360
MEY	0.279	-0.196	0.124	-0.299	-0.014
$IWUE_{ME}$	-0.113	0.419	0.876	-0.047	-0.063
LSR	-0.293	-0.107	-0.005	-0.121	0.155
PLH	0.292	-0.086	-0.134	-0.161	-0.281
ADF	0.269	0.246	-0.057	0.057	0.210
NDF	0.295	-0.064	0.097	0.384	-0.006
HCEL	0.112	-0.480	0.255	0.584	-0.324
Ash	-0.278	-0.198	0.065	-0.196	-0.309
DOM	-0.269	-0.248	0.060	-0.059	-0.153
CPC	-0.247	-0.273	0.072	0.256	0.670
ME	-0.269	-0.246	0.059	-0.077	-0.199
RFV	-0.298	-0.063	-0.058	-0.214	-0.027
Eigenvalue	10.956	3.304	0.363	0.177	0.153
Proportion (%)	73.0	22.1	2.4	1.2	1
Cumulative (%)	73.0	95.1	97.5	98.7	99.7

DMY – dry matter yield, DOMY – digestible organic matter yield, CPY – crude protein yield, MEY – metabolizable energy yield, $IWUE_{ME}$ – irrigation water-use efficiency in metabolizable energy production, LSR – leaf-to-stem ratio, PLH – plant height – ADF – cellulose and lignin, NDF – cellulose, hemicellulose, and lignin, HCEL – hemicellulose, DOM – digestible organic matter, CPC – crude protein content, ME – metabolizable energy content, RFV – relative feed value.

2023). Stomatal closure under drought reduces CO_2 uptake, hindering photosynthesis (Stefanov et al., 2023). Drought stress leads to an accumulation of abscisic acid (ABA), which further induces stomatal closure and inhibits photosynthetic activity (Golzardi et al., 2017). This stress condition also disrupts the water balance within plant cells, leading to cellular dehydration and impaired metabolic functions (Chaves et al., 2009). Additionally, prolonged drought conditions and sub-optimal irrigation can lead to oxidative stress in plants, resulting in the accumulation of reactive oxygen species (ROS). These ROS can damage cellular structures, including membranes, proteins, and DNA, further compromising plant growth and forage yield (Mittler, 2002). The antioxidant defense mechanisms in plants, although activated under stress, may not always be sufficient to counteract the damage caused by ROS, leading to reduced forage yield (Gill and Tuteja, 2010).

Selecting suitable irrigation methods can mitigate water deficit stress effects on sorghum forage production (Ghalkhani et al., 2023). Forage yield was impacted by irrigation methods, with FFI and AFI causing declines compared to CFI. The use of FFI and AFI methods can induce drought stress in half of the plant's root system, reduce the uptake rate of nutrients and water, and subsequently cause a decrease in the photosynthesis rate and then a drop in crop yield (Iqbal et al., 2020; Hosseini et al., 2022; Baghdadi et al., 2023). Mechanistically, reduced water availability in FFI and AFI systems leads to partial root zone drying, which can trigger drought signaling pathways even in well-watered parts of the root system (Davies et al., 2002). This causes a systemic reduction in stomatal conductance and photosynthetic rate, as well as reduced nutrient uptake efficiency (Golzardi et al., 2017; Farhadi et al., 2022). Under partial root drying, plants may also prioritize root growth over shoot growth, leading to reduced biomass accumulation above ground (Kang et al., 2000). However, AFI has been noted to be more effective under water shortage conditions due to enhanced root depth and a broader, more balanced distribution of plant roots, which allows for better water and nutrient acquisition from deeper soil layers (Golzardi et al., 2017).

Speedfeed demonstrated higher forage yield than Pegah, particularly under drought stress, consistent with findings suggesting hybrids' superiority under abiotic stress conditions (Ghalkhani et al., 2023; KhokharVoytas et al., 2023). This superiority is often attributed to better physiological and biochemical adaptations, such as more efficient water use, better osmotic adjustment, and enhanced antioxidant enzyme activities (Blum, 2011).

4.2. IWUE for energy production

The study aligns with prior research, showing increased IWUE of sorghum under drought stress, indicative of drought-tolerant crop adaptability (Kaplan et al., 2019; Farhadi et al., 2022; Baghdadi et al., 2023). Under water-

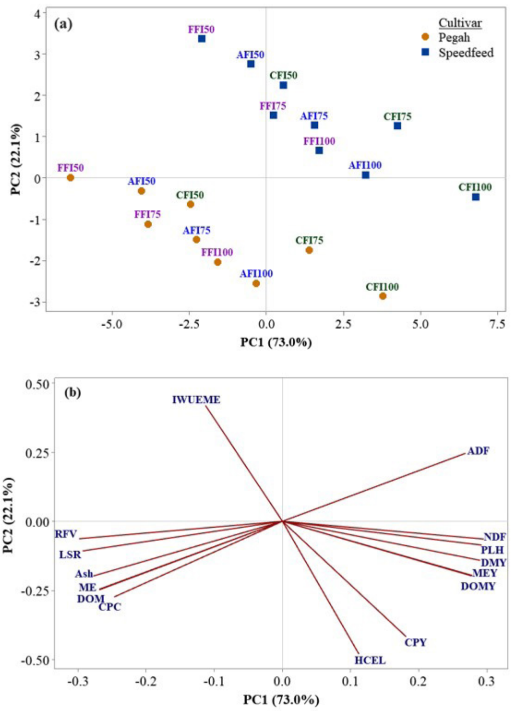

Figure 3. Principal component analysis observation plots (a) and biplot (b) as performed on the forage yield, feed value, and $IWUE_{ME}$ of sorghum affected by irrigation method, drought stress, and cultivar; Explanation as in Tables 4 and 8.

limited conditions, stomatal closure is a primary response that reduces transpiration, enhancing water utilization and IWUE (Yang et al., 2021). This reduction in transpiration minimizes water loss and helps maintain plant water status, thus improving the efficiency of water use relative to biomass production (Ghalkhani et al., 2023). Sorghum responds to drought by enhancing root physiology and architecture, including increased root density and depth, which allows the plant to access water from deeper, less evaporative soil layers (Rajarajan et al., 2021; Baghdadi et al., 2023). This adaptation not only ensures a more stable water supply but also improves the plant's ability to extract nutrients, further supporting growth under drought conditions.

The AFI and FFI methods demonstrated superior $IWUE_{ME}$ compared to CFI, emphasizing the importance of selecting appropriate irrigation techniques for optimal energy output per unit of water. The effectiveness of AFI in enhancing IWUE increased under more severe water deficit conditions, corroborating previous research findings (Golzardi et al., 2017; Baghdadi et al., 2023). Mechanistically, PRD methods, including AFI and FFI, enhance IWUE by creating a heterogeneous soil moisture environment that induces several physiological changes. These methods reduce evaporation and preserve soil moisture, which is critical for maintaining plant water status during drought (Kang et al., 2000). PRD methods also induce morphological and physiological changes in roots, such as increased root-to-shoot ratio, which enhances the plant's ability to absorb water efficiently (Davies et al., 2002). A notable physiological response to PRD is the increased synthesis of ABA in roots exposed to drying soil (Yang et al., 2010; Jia et al., 2014; Iqbal et al., 2020). As a result, the plant minimizes water loss to maximize IWUE while selectively restricting water absorption from the dry root zone and absorbing enough moisture from the wet soil zone (Hosseini et al., 2022; Aghajani et al., 2023). Furthermore, PRD can stimulate the expression of aquaporins, which are proteins that facilitate water transport across cell membranes, enhancing water uptake efficiency (Sade et al., 2012). This physiological adaptation allows the plant to maintain higher IWUE even under reduced irrigation conditions. Optimizing irrigation methods and improving IWUE are crucial for maintaining crop production with limited water availability. Implementing AFI and FFI methods can enhance the resilience of forage crops like sorghum to drought stress, leading to more stable forage yields and better resource utilization. This is particularly important in arid and semi-arid regions where water scarcity poses a major challenge.

4.3. Morphological characteristics

In the present study, the AFI and FFI methods reduced PLH while increasing LSR, thereby enhancing the quality of sorghum forage (Kaplan et al., 2019; Jahanzad et al., 2013). These irrigation methods induce controlled water stress, which triggers physiological responses and hormonal regulation mechanisms, ultimately leading to reduced shoot growth (Iqbal et al., 2020). The primary mechanism involves the modulation of growth hormones such as ABA, which increases under water stress conditions and plays a significant role in inhibiting stem elongation and promoting root growth (Zhang et al., 2006). Increased LSR may result from resource allocation favoring leaf growth over stem growth. Under drought conditions, sorghum tends to allocate more resources to leaf production to optimize photosynthetic capacity and IWUE (Jahanzad et al., 2013). This shift in resource allocation is also influenced by changes in cytokinin levels, which promote cell division in leaves and are reduced in stems under water stress, thus enhancing LSR (Peleg and Blumwald, 2011).

Drought stress reduces PLH while increasing LSR, reflecting plant adaptation to water scarcity (Kaplan et al., 2019; Ghalkhani et al., 2023). Sorghum allocates resources to leaf production during water scarcity, optimizing photosynthetic capacity (Jahanzad et al., 2013). The reduction in stem growth under drought conditions can also be attributed to decreased gibberellin activity, which is essential for stem elongation (Yang et al., 2010). Furthermore, the synthesis of ethylene, a hormone associated with stress responses, can inhibit stem growth, thus contributing to reduced PLH (Sharp and LeNoble, 2002). These physiological mechanisms enable sorghum to optimize growth under water-limited conditions, enhancing forage quality and IWUE (Ghalkhani et al., 2023).

Hybrid Speedfeed consistently exhibits a higher PLH, while cultivar Pegah compensates with a higher LSR, showcasing the adaptability and plasticity of sorghum growth patterns (Jahanzad et al., 2013). These varietal differences underscore the genetic variability within sorghum, emphasizing the importance of selecting appropriate varieties based on specific objectives and growing conditions (Jia et al., 2022; KhokharVoytas et al., 2023). The genetic basis for these differences can be linked to variations in hormone sensitivity and root architecture, which influence the plant's ability to cope with water stress and resource allocation (Blum, 2011).

4.4. Forage quality

The study demonstrates that irrigation management and variety selection can influence the nutritional composition of sorghum, which is crucial for livestock nutrition and digestive health (Bakhtiyari et al., 2020). The findings suggest that drought stress and PRD methods can enhance the nutritional quality of sorghum. Under drought-stress conditions, sorghum plants exhibit physiological responses that resulted in changes in nutrient allocation and the accumulation of specific metabolites (Jahanzad et al., 2013). Increased CPC and ME under water deficit align with prior findings (Farhadi et al., 2022; Pourali et al., 2023), possibly due to enhanced protein synthesis and carbohydrate metabolism changes (Merewitz et al., 2011). One mechanism is the upregulation of stress-responsive proteins and enzymes involved in osmoprotection and cellular repair, which increases protein synthesis (Yamaguchi-Shinozaki and Shinozaki, 2006). Additionally, drought stress induces the accumulation of osmolytes such as proline and soluble sugars, which not only protect cellular structures but also contribute to increased energy availability (Verslues and Sharma, 2010).

Drought stress and alternate furrow irrigation methods impact the components of plant cell walls, such as ADF and NDF (Baghdadi et al., 2023). Under drought conditions, sorghum allocates resources to essential metabolic processes rather than structural fiber production, leading to decreased ADF and NDF (Farhadi et al., 2022). The reduction in structural fibers can be attributed to a shift in carbon allocation from cell wall biosynthesis to the synthesis of soluble carbohydrates and protective compounds (Munné-Bosch and Alegre, 2004). Drought stress also affects carbohydrate metabolism, leading to higher levels of soluble carbohydrates like sugars and starches (Huang et al., 2023). These soluble carbohydrates are more accessible to digest and provide a higher energy content, increasing ME (Baghdadi et al., 2023). By reducing ADF and NDF, the overall RFV improves under drought stress, indicating enhanced digestibility and nutrient availability, making it a more valuable forage source (Bakhtiyari et al., 2020; Pourali et al., 2023).

Improving the feed value of sorghum forage under drought stress is also associated with increased LSR (Jahanzad et al., 2013; Kaplan et al., 2019). An increase in the LSR has been demonstrated to decrease ADF and NDF concentrations while concurrently increasing energy and protein content, thereby improving forage digestibility (Ghalkhani et al., 2023). Since sorghum leaves possess a higher nutritive value relative to stems, any factor that augments the proportion of leaves to stems enhances the overall forage quality (McCuistion et al., 2010; Kaplan et al., 2019). The present study demonstrated that increasing the intensity of drought stress and transitioning the irrigation method from CFI to PRD resulted in an increased LSR, justifying the improvement of forage quality under these conditions (Ghalkhani et al., 2023; Farhadi et al., 2022). Similarly, Bhattarai et al. (2020) demonstrated that deficit irrigation increases the CPC and dry matter digestibility of sorghum forage while simultaneously decreasing its fiber concentration. This research underscores the importance of developing and adopting irrigation techniques that not only conserve water but also maintain or improve crop yield and quality. Consequently, the results contribute to a broader understanding of how to manage water resources more effectively in agriculture, promoting sustainability and food security in regions prone to water deficits.

The study revealed that cultivar Pegah generally exhibited higher DOM, CPC, and ME content compared to the hybrid Speedfeed. These results highlight the importance of selecting suitable sorghum varieties for livestock feed production, as different varieties can exhibit variations in nutritional quality (Jahanzad et al., 2013). The observed differences in feed value between cultivars may be attributed to variations in their genetic makeup, including differences in digestibility, nutrient composition, and anti-nutritional factors (Khalilian et al., 2022). Genetic factors influence the expression of key enzymes and metabolic pathways responsible for nutrient biosynthesis and accumulation, impacting the overall nutritional profile of the forage (Jahanzad et al., 2013; Khalilian et al., 2022; Ghalkhani et al., 2023).

5. Conclusions

This study indicate that transitioning from the CFI to the PRD enhances forage nutritional value and IWUE across various irrigation regimes and sorghum varieties. While CFI achieved the highest forage yield, the FFI and AFI methods resulted in the highest forage quality and IWUE, respectively. The escalation of drought stress intensity across all irrigation methods and cultivars improved IWUE and forage quality, albeit at the expense of reduced forage yield. The hybrid Speedfeed excelled in dry matter and energy production and IWUE, while the cultivar Pegah was superior in forage nutritional value. The study showed a negative correlation between forage yield and quality. The LSR and PLH were identified as suitable indicators of forage quality and yield, respectively. The study recommends cultivating hybrid Speedfeed under moderate drought stress using the AFI method for optimal forage yield and quality, water conservation, and improved IWUE. In cases of severe water scarcity, addressing 50% of the soil moisture deficit is advisable. Further research is necessary to refine these irrigation strategies and explore their long-term impacts on soil health and crop productivity.

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