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ORIGINAL PAPER

Physiological Changes and Nutritional Value of Forage Clitoria Grown in Arid Agro-Ecosystem as Influenced by Plant Density and Water Deficit

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Abstract

Forage crop productivity has lately reduced in countries located in arid and semi-arid regions worldwide due to intensive consumption and the successive years of drought. This problem is exacerbated by the progress of water scarcity. Thus, the current study is aimed at improving the forage productivity and quality of clitoria as a leguminous fodder crop to be involved in crop rotations under low water supply conditions. As an attempt for facing the drought issue, a two-year (SI and SII) field experiment was conducted to evaluate the influence of irrigation pattern (IP) and plant density (PD) on clitoria morpho-physiological attributes, nutritive value, productivity, and irrigation water-use efficiency (IWUE) in two growth cycles (GCI and GCII). Based on the soil water depletion method, three irrigation patterns of 100% ($IP_{0\%}$, full irrigation), 80% ($IP_{20\%}$), and 60% ($IP_{40\%}$) were applied. The tested plant densities were 33 (PD_{33}), 22 (PD_{22}), and 17 (PD_{17}) plants m^{-2}. Findings revealed that $IP_{0\%} \times PD_{22}$ was the efficient treatment for enhancing the physio-biochemical attributes. However, in SI $IP_{0\%} \times PD_{22}$ statistically at par ($p \geq 0.05$) with $IP_{0\%} \times PD_{33}$, $IP_{20\%} \times PD_{22}$, and $IP_{20\%} \times PD_{17}$ (for chlorophyll content in GCI); $IP_{0\%} \times PD_{17}$ and $IP_{20\%} \times PD_{22}$ (for leaf relative water content in GCII); and $IP_{0\%} \times PD_{33}$, $IP_{20\%} \times PD_{33}$, and $IP_{20\%} \times PD_{22}$ (for cell membrane stability index in GCII). Along the two seasons, $IP_{40\%} \times PD_{33}$ was the potent practice for producing the highest leaf: stem ratio in both GCI (2.07 and 1.78) and GCII (1.18 and 0.96). Under $IP_{40\%}$, PD_{33} treatment recorded the greatest protein content in both GCI (24.1–27.0%) and GCII (21.7–19.5%) of SI and SII equaling PD_{22} in GCII (21.2–18.9%) of both seasons and PD_{17} in both GCI (24.0%) and GCII (21.5%) of SI and GCII (19.3%) of SII. The best aggregate protein yield for SI and SII was obtained under $IP_{20\%} \times PD_{33}$ interaction (1.36 and 1.40 t ha^{-1}) without significant difference ($p \geq 0.05$) with $IP_{0\%} \times PD_{33}$ or $IP_{40\%} \times PD_{33}$ interactions. The greatest aggregate dry forage yield was observed in SI under $IP_{0\%}$ or $IP_{20\%}$ combined with PD_{33} (7.77 and 7.52 t ha^{-1}) which did not differ significantly ($p \geq 0.05$). It could be concluded that irrigation by 80% water of full irrigation was found to be an efficient water-saving tactic coupled with adjusting the plant density of 33 plants m^{-2}, which improved clitoria forage quantitative and qualitative properties, in addition to enhancing IWUE. Since leaf relative water content and cell membrane stability index decreased and proline increased in plant tissues under deficit water, clitoria is plant considered a moderately drought tolerant. Thus, clitoria is a promising plant could be successfully grown under arid agro-ecosystems.

Keywords Cell membrane stability · *Clitoria ternatea* · Drought stress · Electrolyte leakage · Forage quality · Plant spacings

1 Introduction

Clitoria (*Clitoria ternatea* L.) is a warm-season and perennial multipurpose legume plant native to tropical America (Ramakrishnan et al. 2018), whose parts contain bioactive compounds for therapeutic purposes (Lijon et al. 2017). Plant regrows well and rapidly after harvesting, high nutritious value, plenteous leaves, and leaves rich in protein. Clitoria forage has low acid digestible fiber, is toxic-free,

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and does not cause bloating. Such advantages make clitoria a beneficial forage plant for hay and silage-making process (Abreu et al. 2014). Moreover, it is utilized for covering and green manuring purposes (Gomez and Kalamani 2003). Clitoria roots can fix atmospheric N_2 for improving soil fertility and enhancing succeeding crops productivity (Sánchez et al. 2011). It is preferred by ruminants like sheep, goats, and cattle due to its high palatable compared with other legumes as well.

Nowadays, the available amount of freshwater for agricultural purposes is reducing worldwide due to the rapid growth of the world's population, numerous human activities, and successively drought occurrence due to climate changes (World Bank 2006). Indeed, forage crop production has currently been reduced in some countries located in arid and semi-arid areas, including Egypt (Saudy 2015), due to prevailing drought conditions. Additionally, reduced feed quantity and quality besides the weak genetic potential are significant factors that directly affect ruminant performance (Descheemaeker et al. 2010). Soil moisture level and planting density are two important factors contributing to crop productivity, including clitoria (Stanisavljević et al. 2012; Jahanzad et al. 2013; Mahfouz et al. 2020; Saudy et al. 2020; Saudy et al. 2021a; Shaaban et al. 2022). Certainly, water availability, beside nutrients, plays a critical role in the success of crop plant cultivation by affecting the growth, productivity, and relevant nutritive features (Testa et al. 2011; Saudy et al. 2022a). Also, water scarcity plays a substantial role in the prevalence of forage plants across varied habitats by affecting their growth and yield stability worldwide (Shao et al. 2009; Rostamza et al. 2011). Globally, irrigation water scarcity has become a global serious issue, particularly in arid and semi-arid agroecosystems, since crop production system mainly depends on irrigation (Wei et al. 2016; El-Metwally and Saudy 2021a); El-Bially et al. 2022a; El-Metwally et al. 2022a). Irrigation water also is a critical factor in food and feed production and a vital tool for guaranteeing food security and stabilizing socio-economic status in many areas of the world (Liang et al. 2016). The world's agricultural sector consumes about 80% of available water resources (FAO-Aquastat 2015; Abdelhafez et al. 2020). Therefore, optimizing the efficiency of irrigation water use without decreasing quantitative and qualitative crop traits has become an imperative strategy (Pereira et al. 2012; El-Metwally et al. 2021; El-Bially et al. 2022b; Saudy et al. 2023a). Recently, how to produce more crops per drop of water under the limited water supply is a challenge for irrigated agriculture worldwide. Improving irrigation water management, particularly at the field scale, is considered the adopted practice among the manners of confronting this challenge. The integrative effect of deficit irrigation strategy with achieving and maintaining the optimal plant density of the clitoria plant appears to be a very effective practice to actualize this goal (Jahanzad et al. 2013).

In arid and semi-arid regions, deficit irrigation usually has been applied for irrigation water saving and maximizing water productivity (Shahrokhnia and Sepaskhah 2016; Mahfouz et al. 2020). The main objective of applying deficit irrigation is to obtain maximum irrigation utilization and stable production instead of achieving the greatest yields (Kirda 2002; Fereres and Soriano 2007). Moreover, this strategy can else have further benefits including decreasing the energy utilized during the irrigation process, reducing nitrate loss by leaching, improving nutrients utilization, enhancing the competitive ability economic crops (Falagán et al. 2015; El-Metwally and Saudy 2021b; Mubarak et al. 2021; Salem et al. 2021; Abd–Elrahman et al. 2022; Saudy and El-Metwally 2023), and reducing agricultural production costing and water consumption (Pulupol et al. 1996; El-Bially et al. 2018).

Plant population density is an important factor related to quantitative and qualitative parameters in forage plants (Mattera et al. 2013; Saudy and El-Bagoury 2014; Ramanjaneyulu et al. 2018). Increasing planting density has been mentioned to be effective in intercepting the solar radiation (Saudy and El-Metwally 2009; Saudy 2013) and therefore degree of dry matter accumulating in forage legumes (Purcell et al. 2002), improving forage yield with high nutritional value (Seiter et al. 2004). Therefore, choosing the plant density is depending on the hypothesis that the optimal density enables the plant canopy to intercept the fully photosynthetically active radiation, resulting in a higher yield. This response was noted in various crops such as soybean (Andrade et al. 2002), lucerne (Mattera et al. 2013), and cowpea (Kamara et al. 2018). In this respect, clitoria also might be a very promising legume forage plant that can grow in arid and semi-arid agroecosystems. However, little knowledge is available about the influence of irrigation pattern plus planting densities on performance and irrigation water-use efficiency (IWUE) of clitoria till now.

Our study hypothesis was that deficit irrigation could interact with high plant density producing comparable clitoria productivity as that of optimal irrigation. Bearing the above in mind, the objective of this study is to provide useful information about the impact of irrigation patterns and different planting densities on morpho-physiological attributes, nutritive value, productivity, and IWUE of clitoria under arid agroecosystem conditions.

2 Materials and Methods

2.1 Experimental Site Description

A 2-year field experiment was carried out at the research farm of the Faculty of Agriculture which is located in southeast El-Fayoum governorate, Egypt (29.17° N; 30.53° E), during 2015 and 2016. According to the aridity categorization (Ponce et al. 2000), the climate is typical of arid areas. Herein, the location's climate during the period of study (i.e., May to September) is commonly summery and arid with no rainfall. The averages of climatic data were 37.54 °C and 38.56 °C for day temperatures 22.54 °C and 23.10 °C for night temperatures, 38.34% and 34.92% for relative humidity, and 6.84 and 6.48 mm d^{-1} for class "A" pan evaporation in both seasons, respectively. Before sowing, soil samples were collected each 20 cm to a maximum depth of 60 cm (0–20, 20–40, and 40–60 cm). Hence, the basal physical and chemical properties of the experimental soil were determined using the standard methods described by Page et al. (1982) and Klute (1986). The analysis proved that the soil of the experimental site was classified as sandy-loam comprising 73.8% sand, 13.9% silt, and 12.3% silt as well as having 0.79% organic matter, 6.53% calcium carbonate, 7.85 pH, 3.40 dS m^{-1} electric conductivity, 13.73 mg kg^{-1} total nitrogen, 3.26 mg kg^{-1} available phosphorus, and 40.57 mg kg^{-1} available potassium. In the root zone, soil water contents at the field capacity and permanent wilting point were 19.3 and 4.9%, respectively, across the three layers. Mean bulk density varies from 1.46 to 1.58 g cm^{-3}.

2.2 Experimental Design and Treatments

The experiment was a split plot in randomized complete block design (RCBD) using three replications, involving three irrigation patterns (IP), distributed in the main plots, and three planting densities (PD), allocated to the sub-plots. The amounts of irrigation water applied (IWA) were implemented based on allowed soil water depletion (ASWD). Irrigation treatments were involved irrigating with 100, 80, and 60% of ASWD ($IP_{0\%}$, $IP_{20\%}$, and $IP_{40\%}$). According to Allen et al. (1998), ASWD was presumed to be 50 ± 2% under no water stress conditions in the effective rooting depth. Planting density treatments were applied by adjusting three intra-ridge spacings of 10, 15, and 20 cm achieving PD of 33 (PD_{33}), 22 (PD_{22}), and 17 (PD_{17}) plants m^{-2}, respectively. The net area of each subplot (experimental unit) was 12 m^2; 4 m in length × 3 m in width, consisting of five ridges 0.6 cm apart. One ridge of each side comprised the border to eliminate edge effects, while the remaining three central ridges were utilized to take samples and appreciations.

2.3 Irrigation Scheming

All experimental units received the same amounts of irrigation water until clitoria plants fully emerged (21 days after sowing, DAS). IP treatments were applied using a surface irrigation system. To determine the actual irrigation water amount, the soil water content was monitored by the gravimetric procedure as described by Smith and Warrick (2007) and was checked and confirmed by using the digital W.E.T. sensors (Moisture Meter type HH2, Cambridge, CB5 0 EJ, UK). Once IP treatments commenced, the soil water content was measured at 0.2 m increments down to 0.6 m, using a gravimetric method for well-watered ($IP_{0\%}$) plants before 2-day intervals of each irrigation. In the oven-dried at 105 °C, these soil samples instantly were dried for 24 h. The percent of ASWD was calculated from all soil layers in the effective rooting depth by Eq. (1) outlined by Martin et al. (1990).

$$D\,(\%) = 100 \times \frac{1}{n}\sum_{i=1}^{n}\left[\left(FC_i - \theta_i\right)/FC_i - PWP_i\right] \quad (1)$$

whereas $D\%$ is the percent of depleted soil water, FC_i is the gravimetric soil water for i^{th} layer at field capacity point, θ_i is the gravimetric soil water in i^{th} layer, PWP_i is the gravimetric soil water for i^{th} layer at permanent wilting point, and n is the soil layers number of the effectual rooting zone.

The maximum ASWD for clitoria without water deficit was presumed to be 50% (Allen et al. 1998) of the total available soil water under no water deficit conditions was utilized as a control level ($IP_{0\%}$) and the other IP levels received a percentage, i.e., 80% and 60% for $IP_{20\%}$ and $IP_{40\%}$ levels, respectively, of the volume of IWA at full irrigation ($IP_{0\%}$). So irrigation started when 50±2% of total available water in the rooting zone was depleted and the actual IWA required at each irrigation was computed using Equation (2).

$$\text{IWA} = \left[\left(\left(\text{FC}_i - \theta_i\right) \times D \times \text{A}\right)/\left(100 \times E_i\right)\right] \quad (2)$$

where IWA is in m^3, FC_i is the gravimetric soil water for i^{th} layer at field capacity point measured after 24 h of water application, θ_i is the gravimetric soil water just before irrigation in i^{th} layer, D is the effective rooting zone (m), A is the subplot area (m^2), and E_i is the efficiency of irrigation taken as 0.6 (Howell 2003).

The IWA amount delivered for each subplot was controlled by a plastic water pipe of 5 cm diameter. Each subplot is equipped with a water pipe to deliver water from the field's waterway. The actual volume of water transported through water pipes was calculated by Eq. (3) reported by Israelsen and Hansen (1962).

$$Q = CA\sqrt{2gh} \times 10^{-3} \quad (3)$$

where Q is the discharge of water (L s^{-1}), C is the coefficient of discharge, A is the water-pipe cross-section area (cm^2), h is the effectual head of irrigation water above the water-pipe tip (cm), and g is the acceleration of gravity (cm s^{-2}).

The main plots allotted to IP treatments were isolated with 2 m fallow land borders to keep off the laterally subsurface transition of water from one to another. As well, neighboring experimental plots within each IP treatment were separated by fallow land 0.6 m in width. The total IWA under $IP_{0\%}$, $IP_{20\%}$, and $IP_{40\%}$ were 5279.0–5659.0, 4318.4–4527.2, and 3238.8–3395.4 $m^3\ ha^{-1}$ added through 9 and 10 irrigations in SI and SII, respectively.

2.4 Crop Husbandry

Clitoria cv. Baladi seeds were introduced from Sudan and were treated with *Rhizobium* sp. of the cowpea group according to (Abreu et al. 2014) before sowing to fix atmospheric N_2. Five healthy seeds were planted in hills on 20th May in SI and SII. At 15 DAS, clitoria seedlings were thinned to 2 plants per hill. During seedbed preparation, 31 kg ha^{-1} of P_2O_5 and 50 kg ha^{-1} of K_2O were added for all subplots. By checking the plant roots 30 DAP, we found that clitoria plants failed to form root nodulations. Thence, clitoria fertilized by 30 kg N in each growth cycle (GC) portioned into two equal applications before the first and third irrigations in GCI and applied after harvesting before the first two irrigations in GCII in both seasons. Weed control was done by hand pulling when required.

2.5 Sampling and Measurements

During each season, two consecutive growth cycles or cuts (GCI and GCII) of clitoria were harvested. The GCI clipped at 60 DAS while the GCII was obtained 105 DAS. All cuts were harvested manually by mower at a uniform height of about 10±2 cm above the soil surface. A sample of six plants was randomly to estimate the following:

2.5.1 Physio-Biochemical Attributes

Total chlorophyll (Arnon (1949)), leaf relative water content (Weatherley 1950), cellular membrane stability index (Premachandra et al. 1990), ion leakage (Lutts et al. 1996), and proline content (Bates et al. 1973) were determined. Furthermore, the absolute growth rate between two different times in each GC was calculated based on the dry matter accumulation by using Equation (4) given by Hunt (1990).

$$\text{Absolute growth rate}\ \left(\text{g d}^{-1}\right) = \left[\left(W_2 - W_1\right)/\left(T_2 - T_1\right)\right] \quad (4)$$

where W_1 and W_2 refer to dry weight per plant (g) at the first (T_1) and second (T_2) sampling in (d) for each GC, respectively.

2.5.2 Morphological Attributes

The measured morphological attributes were plant height, leaf number plant^{-1}, branch number plant^{-1}, dry weight plant^{-1}, leaf: stem ratio based on fresh mass, and leaf area index (Watson and Watson 1953).

2.5.3 Nutritive Value Indices

To determine the nutritive value indices, powdered dry samples of foliage parts (leaves and stems) were taken from each experimental plot in each GC for forage analysis during the two seasons. The nutritive value of forage clitoria (protein, fiber, and non-structural carbohydrates percentages) was measured by near-infrared reflectance spectroscopy appliance produced by FOSS Allé 1 in Denmark (AOAC 2012).

2.5.4 Forage and Protein Yields and Irrigation Water Use Efficiency

The internal two ridges of each experimental plot (4.80 m^2) were harvested to appreciate the yield of fresh forage, then samples of this fresh forage were dried at 70°C till constant, and dry matter percentage was estimated, and then the yield of dry forage was computed. The aggregate dry forage yield and aggregate protein yield (gross yield of both GCI and GCII) were calculated. Moreover, irrigation water-use efficiency (IWUE) was calculated using Equation (5) as proposed by Jensen (1983).

$$\text{IWUE}\ \left(\text{kg m}^{-3}\right) = \left[\text{dry forage yield}\ \left(\text{kg ha}^{-1}\right)/\text{applied water}\ \left(\text{m}^3\ \text{ha}^{-1}\right)\right] \quad (5)$$

2.6 Statistical Analysis

All recorded data during the two seasons were analyzed following the technique of analysis of variance for split plot arranged in randomized complete block design using three replications. Seasons, IP, and PD were considered as fixed effects while replications (blocks) were considered random effects. Genstat Software computer software package. Before analysis of variance, each attribute was explored for normal distribution agreeing to the test of Shapiro-Wilk (with confidence level $p \leq 0.05$ for significant difference from a normal distribution). Most of the attributes were in normal or close to the normal distribution. The differences among the means were compared by Duncan's test at $p \leq 0.05$.

Forage yield and IWUE data were correlated with the amount of irrigation water applied. According to the following linear model (Equation (6)) correlation was performed at a significance level of a=0.05 using STATGRAPHICS Centurion XVI.

$$y = a + bx \quad (6)$$

where y is dry forage yield or IWUE data, x is the amount of irrigation water applied data, a is the intercept, and b is the slope of the regression line.

3 Results

3.1 Physio-Biochemical Attributes

The interaction of IP and PD had significant ($p \leq 0.05$) effects on all clitoria's physio-biochemical attributes, except chlorophyll content in GCII of SI, leaf relative water content in GCII of SII, cell membrane stability index in GCI of SI, and ion leakage in both GCI and GCII of the SI and SII (Tables 1 and 2). Generally, $IP_{0\%} \times PD_{22}$ was the efficient treatment for enhancing the physio-biochemical attributes. However, in SI such potent treatment statistically at par ($p \geq 0.05$) with $IP_{0\%} \times PD_{33}$, $IP_{20\%} \times PD_{22}$, and $IP_{20\%} \times PD_{17}$ (for chlorophyll content in GCI); $IP_{0\%} \times PD_{17}$ and $IP_{20\%} \times PD_{22}$ (for leaf relative water content in GCII); and $IP_{0\%} \times PD_{33}$, $IP_{20\%} \times PD_{33}$ and $IP_{20\%} \times PD_{22}$ (for cell membrane stability index in GCII). Also, at GCI of SII, the obtained values of chlorophyll content (with $IP_{20\%} \times PD_{17}$), leaf relative water content (with $IP_{0\%} \times PD_{17}$, $IP_{20\%} \times PD_{33}$, and $IP_{40\%} \times PD_{22}$), and cell membrane stability index (with $IP_{0\%} \times PD_{33}$ and $IP_{20\%} \times PD_{33}$) were similar to that of $IP_{0\%} \times PD_{22}$ ($p \geq 0.05$). In both GCI and GCII of SI and SII, $IP_{0\%} \times PD_{17}$ recorded the highest value of absolute growth rate without remarkable variation ($p \geq 0.05$) with $IP_{20\%} \times PD_{17}$ in GCII of SI and GCI of SII (Table 2). On the contrary, $IP_{40\%} \times PD_{17}$ in both GCI a GCII of SI and SII, in addition to $IP_{40\%} \times PD_{22}$ in GCII of SI showed the maximum values ($p \leq 0.05$) of proline content.

3.2 Morphological Attributes

Clitoria plants differed significantly ($p \leq 0.05$) in their response to the interaction effect between IP and PD for all morphological attributes, except plant height and branch number plant^{-1} in GCI and leave number plant^{-1} in GCII during SI as well as plant height, dry weight plant^{-1}, and leaf area index in GCII during SII (Tables 3 and 4). In the first season, the most distinctive combinations ($p \leq 0.05$) for enhancing plant growth were $IP_{0\%} \times PD_{33}$ or $IP_{20\%} \times PD_{22}$ (for plant height in GCII) as well as $IP_{0\%} \times PD_{17}$ (for leaf number plant^{-1} in GCI) and $IP_{20\%} \times PD_{17}$ (for branch number plant^{-1} in GCII). In the second season, plant height showed the maximum increase with $IP_{0\%} \times PD_{33}$ in GCI. While $IP_{0\%} \times PD_{17}$ was the

Table 1 Effect of irrigation pattern and planting density interaction on total chlorophyll, leaf relative water content, and cell membrane stability index for both growth cycles (GCI and GCII) of clitoria in 2015 (SI) and 2016 (SII)

Irrigation	Planting density	Chlorophyll content (mg g^{-1})		Leaf relative water content (%)		Cell membrane stability index	
		GCI	GCII	GCI	GCII	GCI	GCII
		SI					
$IP_{0\%}$	PD_{33}	14.7±0.3ab	11.5±0.4a	72.0±0.7bc	62.5±1.6b	86.4±0.7a	50.6±2.7a
	PD_{22}	15.2±0.2a	11.7±0.6a	79.9±1.1a	80.7±1.8a	86.8±2.3a	48.2±2.7ab
	PD_{17}	13.2±0.2cd	13.7±0.6a	74.3±0.9b	77.5±1.0a	80.4±0.7a	43.3±2.3bcd
$IP_{20\%}$	PD_{33}	13.9±0.2bc	11.7±0.1a	74.6±1.0b	57.4±1.0c	83.4±1.6a	45.1±2.7abc
	PD_{22}	14.5±0.2ab	12.3±0.4a	74.7±0.3b	76.0±1.5a	82.2±0.8a	46.8±3.2abc
	PD_{17}	14.4±0.1ab	12.5±0.5a	75.7±1.4b	63.1±1.1b	69.0±2.6a	38.9±1.9d
$IP_{40\%}$	PD_{33}	12.7±0.3d	11.9±0.6a	70.5±0.7c	51.9±1.3d	79.7±1.8a	41.8±5.6cd
	PD_{22}	13.9±0.6bc	11.3±0.3a	72.7±1.4bc	67.4±2.3b	75.4±1.0a	38.2±1.9d
	PD_{17}	13.1±0.1cd	12.1±0.3a	62.4±2.3d	57.1±1.1c	72.2±4.5a	41.9±2.6cd
		SII					
$IP_{0\%}$	PD_{33}	14.7±0.2e	15.8±0.1c	66.4±0.9c	83.8±0.8a	68.4±4.1a	83.0±1.3a
	PD_{22}	18.4±0.3a	20.7±0.3a	73.9±0.8a	86.2±0.4a	62.4±4.7ab	73.8±1.7cd
	PD_{17}	17.1±0.3bc	16.5±0.8c	70.4±0.7ab	83.2±2.4a	43.1±1.3ef	78.3±2.0bc
$IP_{20\%}$	PD_{33}	14.9±0.3e	16.0±0.7c	71.3±1.1ab	85.4±0.8a	56.2±0.9bc	81.4±1.1ab
	PD_{22}	15.6±0.1de	18.9±0.5b	66.5±1.4c	81.8±2.3a	54.9±3.4cd	74.0±3.0cd
	PD_{17}	17.9±0.6ab	17.8±0.3b	66.5±1.2c	80.7±1.3a	43.1±2.3ef	64.0±1.4f
$IP_{40\%}$	PD_{33}	15.5±0.5de	16.3±0.3c	66.1±0.3c	80.3±1.2a	54.4±1.2cd	67.1±0.8ef
	PD_{22}	16.1±0.1cd	15.9±0.3c	71.5±0.5ab	80.4±1.7a	48.4±3.2de	70.3±0.9df
	PD_{17}	15.6±0.3de	13.9±0.3d	68.7±0.5bc	76.7±2.7a	36.9±1.8f	71.8±0.2d

$IP_{0\%}$, $IP_{20\%}$, and $IP_{40\%}$: irrigation by 100, 80, and 60% of allowed soil water depletion, respectively. PD_{33}, PD_{22}, and PD_{17}: planting density of 33, 22, and 17 plants m^{-2}, respectively. Means within the same column of the same season followed by the same letter are not significantly different at $p \leq 0.05$ according to Duncan's test

Table 2 Effect of irrigation pattern and planting density interaction on ion leakage, proline content, and absolute growth rate for both growth cycles (GCI and GCII) of clitoria in 2015 (SI) and 2016 (SII) seasons

Irrigation	Planting density	Ion leakage (%)		Proline content (mg g^{-1} DW)		Absolute growth rate (g d^{-1})	
		GCI	GCII	GCI	GCII	GCI	GCII
		SI					
$IP_{0\%}$	PD_{33}	53.0±3.3a	44.5±1.1a	3.30±0.04cd	0.54±0.02d	0.16±0.01d	0.55±0.01d
	PD_{22}	50.3±2.9a	56.0±0.4a	4.70±0.14b	0.63±0.01d	0.21±0.01c	0.68±0.01c
	PD_{17}	56.6±2.8a	47.9±0.1a	2.90±0.25de	1.14±0.03bc	0.31±0.01a	0.94±0.01a
$IP_{20\%}$	PD_{33}	62.9±1.1a	49.2±4.4a	2.70±0.15e	1.23±0.03b	0.13±0.01ef	0.63±0.01c
	PD_{22}	66.1±4.6a	51.8±1.9a	3.60±0.11c	1.22±0.04b	0.25±0.02b	0.84±0.04b
	PD_{17}	74.7±1.2a	50.4±2.1a	3.70±0.14c	1.07±0.01c	0.26±0.01b	0.89±0.03ab
$IP_{40\%}$	PD_{33}	73.0±4.0a	58.4±1.4a	4.30±0.11b	1.12±0.03bc	0.11±0.01f	0.61±0.02cd
	PD_{22}	64.8±1.2a	67.3±2.2a	4.50±0.07b	1.59±0.11a	0.14±0.01de	0.68±0.02c
	PD_{17}	74.6±3.6a	63.5±1.1a	5.10±0.33a	1.64±0.05a	0.21±0.01c	0.81±0.03b
		SII					
$IP_{0\%}$	PD_{33}	76.5±0.2a	84.5±0.6a	1.50±0.02cd	0.81±0.01f	0.11±0.01d	0.46±0.02c
	PD_{22}	57.6±1.4b	88.5±2.9a	1.54±0.07cd	0.81±0.01f	0.22±0.01b	0.55±0.04bc
	PD_{17}	75.7±3.4a	83.9±1.8a	1.38±0.11d	1.03±0.02d	0.26±0.02a	0.72±0.03a
$IP_{20\%}$	PD_{33}	75.4±4.6a	87.4±2.6a	1.63±0.05c	0.98±0.01e	0.12±0.01d	0.55±0.02bc
	PD_{22}	80.7±5.9a	79.6±0.6a	1.45±0.03cd	1.16±0.05b	0.20±0.01b	0.47±0.04c
	PD_{17}	82.9±2.8a	82.9±4.1a	1.41±0.03d	1.07±0.01cd	0.26±0.01a	0.55±0.04bc
$IP_{40\%}$	PD_{33}	82.7±1.4a	89.6±1.1a	1.37±0.04d	1.08±0.02c	0.10±0.01d	0.50±0.06c
	PD_{22}	77.5±1.3a	88.4±1.5a	3.67±0.02b	1.05±0.01cd	0.11±0.01d	0.57±0.02bc
	PD_{17}	85.0±3.3a	89.6±1.7a	4.20±0.01a	1.25±0.01a	0.15±0.01c	0.63±0.01ab

$IP_{0\%}$, $IP_{20\%}$, and $IP_{40\%}$: irrigation by 100, 80, and 60% of allowed soil water depletion, respectively. PD_{33}, PD_{22}, and PD_{17}: planting density of 33, 22, and 17 plants m^{-2}, respectively. Means within the same column of the same season followed by the same letter are not significantly different at $p \leq 0.05$ according to Duncan's test

Table 3 Effect of irrigation pattern and planting density interaction on plant height leaf number plant^{-1} and branch number plant^{-1} for both growth cycles (GCI and GCII) of clitoria in 2015 (SI) and 2016 (SII) seasons

Irrigation	Planting density	Plant height (cm)		Leaves number plant^{-1}		Branches number plant^{-1}	
		GCI	GCII	GCI	GCII	GCI	GCII
		SI					
$IP_{0\%}$	PD_{33}	53.9±0.4a	61.5±0.4a	29.4±0.3d	123.8±3.5a	3.3±0.1a	13.8±0.3d
	PD_{22}	48.3±0.1a	55.4±0.8c	35.2±0.1c	147.6±7.3a	3.6±0.1a	14.1±0.4cd
	PD_{17}	41.4±0.4a	50.3±0.9d	41.8±1.5a	190.3±0.6a	4.0±0.1a	20.3±0.3a
$IP_{20\%}$	PD_{33}	54.2±0.8a	59.1±0.8b	26.1±0.1e	116.2±0.9a	2.7±0.1a	13.1±0.5d
	PD_{22}	48.9±1.0a	60.6±0.7ab	39.0±0.6b	151.1±6.0a	3.4±0.2a	16.6±0.6b
	PD_{17}	39.1±1.1a	59.0±0.2b	40.7±0.2ab	177.9±5.6a	3.7±0.1a	19.5±0.7a
$IP_{40\%}$	PD_{33}	45.8±3.1a	54.7±0.4c	25.4±0.3e	110.3±1.3a	2.6±0.1a	11.6±0.3e
	PD_{22}	40.6±1.8a	53.5±0.7c	31.1±0.4d	126.2±1.2a	3.3±0.1a	15.4±0.3bc
	PD_{17}	34.0±1.3a	50.3±1.0d	36.7±0.7c	156.7±6.1a	4.0±0.3a	16.5±0.5b
		SII					
$IP_{0\%}$	PD_{33}	50.6±2.2a	64.3±2.4a	28.0±0.5de	120.4±1.0cd	3.1±0.2efg	13.1±0.5ef
	PD_{22}	40.1±1.4cd	62.2±1.3a	36.2±0.9c	148.1±1.8a	3.5±0.3de	14.5±0.1cd
	PD_{17}	43.2±0.2bc	55.3±1.4a	41.8±0.1a	149.3±1.4a	5.5±0.2a	19.4±0.2a
$IP_{20\%}$	PD_{33}	46.4±1.1b	61.7±1.1a	27.0±0.2ef	119.2±3.5cd	3.7±0.2cd	12.3±0.5f
	PD_{22}	45.0±0.1b	58.1±2.1a	38.7±0.7b	129.5±0.7b	4.1±0.1c	15.0±0.1bc
	PD_{17}	40.7±0.4cd	56.5±0.5a	40.0±1.4ab	144.0±3.5a	4.8±0.1b	16.1±0.4b
$IP_{40\%}$	PD_{33}	38.8±1.0de	59.4±2.2a	25.7±0.1f	112.3±5.2d	2.9±0.1fg	13.2±0.2def
	PD_{22}	38.9±0.7de	54.1±0.9a	27.4±0.2def	126.0±4.4bc	2.8±0.1g	14.9±0.7bc
	PD_{17}	36.3±0.8e	56.3±1.3a	29.6±0.2d	142.8±0.6a	3.4±0.1def	14.3±0.7cde

$IP_{0\%}$, $IP_{20\%}$, and $IP_{40\%}$: irrigation by 100, 80, and 60% of allowed soil water depletion, respectively. PD_{33}, PD_{22}, and PD_{17}: planting density of 33, 22, and 17 plants m^{-2}, respectively. Means within the same column of the same season followed by the same letter are not significantly different at $p \leq 0.05$ according to Duncan's test

Table 4 Effect of irrigation pattern and planting density interaction on dry weight/plant leaves area index and leaf: stem ratio for both growth cycles (GCI and GCII) of clitoria in 2015 (SI) and 2016 (SII) seasons

Irrigation	Planting density	Dry weight plant^{-1} (g)		Leaf area index		Leaf: stem ratio	
		GCI	GCII	GCI	GCII	GCI	GCII
		SI					
$IP_{0\%}$	PD_{33}	3.3±0.1d	19.9±0.2cde	2.4±0.04a	9.5±0.24a	1.57±0.05cd	1.12±0.02abc
	PD_{22}	5.2±0.3b	23.7±0.8b	2.1±0.04b	6.6±0.19c	1.34±0.07fg	1.11±0.03bc
	PD_{17}	6.2±0.1a	26.0±0.3a	1.5±0.02d	5.5±0.03e	1.50±0.08de	1.00±0.03d
$IP_{20\%}$	PD_{33}	3.6±0.1d	19.0±0.1e	1.9±0.08c	9.0±0.42ab	1.42±0.12ef	1.11±0.02bc
	PD_{22}	4.4±0.1c	21.0±0.1c	2.1±0.10b	8.5±0.09b	1.27±0.19g	1.08±0.02c
	PD_{17}	5.8±0.1a	25.7±1.1a	1.5±0.03d	6.1±0.07cd	1.49±0.06de	1.17±0.03ab
$IP_{40\%}$	PD_{33}	2.6±0.1e	19.1±0.2de	1.9±0.09c	8.5±0.13b	2.07±0.06a	1.18±0.02a
	PD_{22}	3.2±0.2d	20.5±0.2cd	1.4±0.07d	5.7±0.03de	1.83±0.07b	1.14±0.01abc
	PD_{17}	4.8±0.1c	22.8±0.3b	1.4±0.02d	4.9±0.01f	1.69±0.04c	1.16±0.02ab
		SII					
$IP_{0\%}$	PD_{33}	3.1±0.1d	20.3±0.1a	1.8±0.09a	10.1±0.17a	1.75±0.02a	0.75±0.02c
	PD_{22}	4.8±0.1b	23.0±0.4a	1.4±0.03c	7.4±0.33a	1.20±0.02e	0.65±0.01d
	PD_{17}	5.5±0.2a	26.2±0.3a	1.2±0.01cde	5.6±0.18a	1.37±0.02d	0.65±0.02d
$IP_{20\%}$	PD_{33}	3.0±0.1d	19.8±0.6a	1.6±0.04b	8.2±0.23a	1.57±0.03c	0.73±0.01c
	PD_{22}	4.2±0.1c	20.8±0.3a	1.2±0.04de	6.3±0.33a	1.44±0.02d	0.76±0.02c
	PD_{17}	5.3±0.2a	24.1±0.6a	1.1±0.02e	4.8±0.23a	1.38±0.03d	0.63±0.02d
$IP_{40\%}$	PD_{33}	2.4±0.1e	19.4±0.7a	1.2±0.02cd	8.1±0.05a	1.78±0.03a	0.96±0.03a
	PD_{22}	2.6±0.1e	21.0±0.1a	0.9±0.03f	5.9±0.22a	1.57±0.01c	0.95±0.03a
	PD_{17}	3.1±0.1d	22.9±0.1a	0.6±0.01g	3.7±0.21a	1.65±0.04b	0.81±0.05b

$IP_{0\%}$, $IP_{20\%}$, and $IP_{40\%}$: irrigation by 100, 80, and 60% of allowed soil water depletion, respectively. PD_{33}, PD_{22}, and PD_{17}: planting density of 33, 22, and 17 plants m^{-2}, respectively. Means within the same column of the same season followed by the same letter are not significantly different at $p \leq 0.05$ according to Duncan's test

effective combination for increasing leaf number plant^{-1} and branch number plant^{-1} in both GCI and GCII, statistically leveled ($p \geq 0.05$) with $IP_{20\%} \times PD_{17}$ (for leaf number plant^{-1} in GCI and GCII) and each of $IP_{0\%} \times PD_{22}$ and $IP_{40\%} \times PD_{17}$ (for leaf number plant^{-1} in GCII). Furthermore, in the first season, $IP_{0\%} \times PD_{17}$ in both GCI and GCII and $IP_{20\%} \times PD_{17}$ in GCII (for dry weight plant^{-1}) as well as $IP_{0\%} \times PD_{33}$ in both GCI and GCII and $IP_{20\%} \times PD_{33}$ in GCII (for leaf area index) recorded the highest significant values ($p \leq 0.05$). In GCI, $IP_{0\%} \times PD_{17}$ and $IP_{20\%} \times PD_{17}$ (for dry weight plant^{-1}) and $IP_{0\%} \times PD_{33}$ (for leaf area index) gave the maximal increases ($p \leq 0.05$) in the second season. Along the two seasons, $IP_{40\%} \times PD_{33}$ was the potent practice for producing the highest leaf: stem ratio in both GCI and GCII, however, significantly leveled ($p \geq 0.05$) with $IP_{0\%} \times PD_{33}$ at GCI in the second season as well as $IP_{0\%} \times PD_{33}$, $IP_{20\%} \times PD_{17}$, $IP_{40\%} \times PD_{22}$, and $IP_{40\%} \times PD_{17}$ in the first season, in addition to $IP_{40\%} \times PD_{22}$ in the second season at GCII.

3.3 Nutritive Value Indices

Data depicted in Figs. 1 and 2 exhibited that the interaction of IP × PD significantly ($p \leq 0.05$) affected all studied forage nutritive value indices for both GCI and GCII in the two seasons. Under $IP_{40\%}$, PD_{33} treatment recorded the greatest

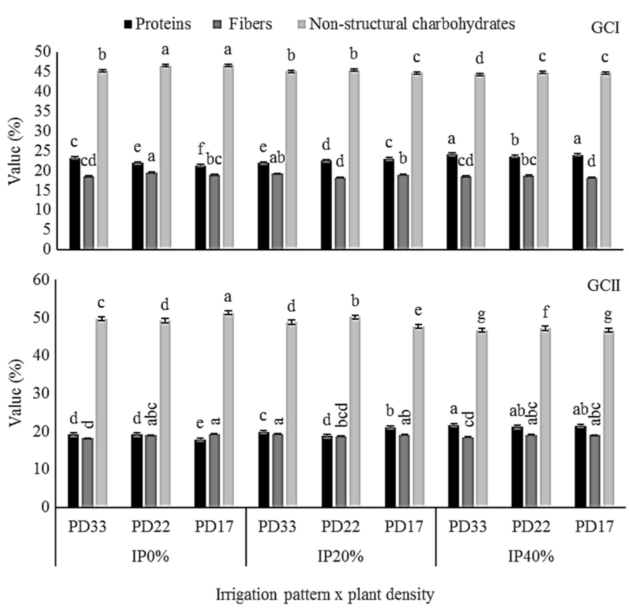

Fig. 1 Effect of irrigation pattern and planting density interaction on proteins, fibers, and non-structural carbohydrates for both growth cycles (GCI and GCII) of clitoria in 2015 season. IP0%, IP20%, and IP40%: irrigation by 100, 80, and 60% of allowed soil water depletion, respectively. PD33, PD22, and PD17: planting density of 33, 22, and 17 plants m^{-2}, respectively. Means within the same bar of the same season followed by the same letter are not significantly different at $p \leq 0.05$ according to Duncan's test

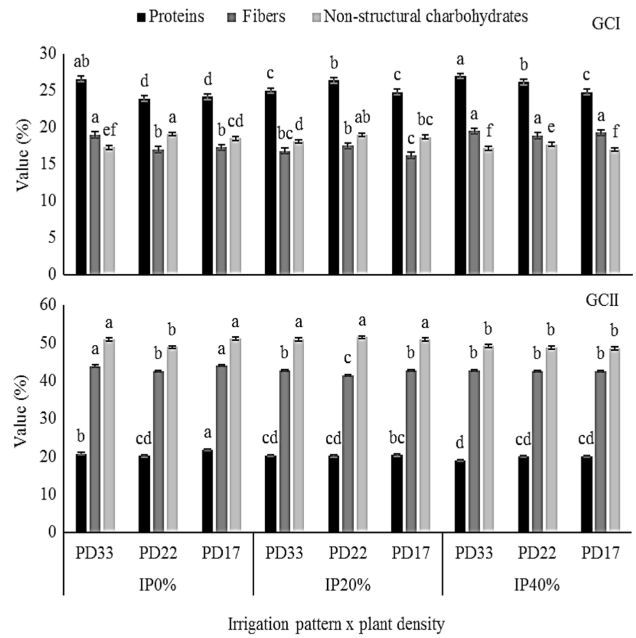

Fig. 2 Effect of irrigation pattern and planting density interaction on proteins, fibers and non-structural carbohydrates for both growth cycles (GCI and GCII) of clitoria in 2016 season. IP0%, IP20%, and IP40%: irrigation by 100, 80, and 60% of allowed soil water depletion, respectively. PD33, PD22, and PD17: planting density of 33, 22, and 17 plants m^{-2}, respectively. Means within the same bar of the same season followed by the same letter are not significantly different at $p \leq 0.05$ according to Duncan's test

protein content in both GCI and GCII of SI and SII equaling PD_{22} in GCII of both seasons and PD_{17} in both GCI and GCII of SI and GCII of SII. Also, $IP_{0\%} \times PD_{33}$ had similar protein content as $IP_{40\%} \times PD_{33}$ in both GCI and GCII of SI. $IP_{0\%} \times PD_{22}$ along $IP_{20\%} \times PD_{33}$ in SI and $IP_{0\%} \times PD_{22}$ in SI for GCI possessed the maximum fiber content. Also, $IP_{0\%} \times PD_{17}$ was the potent treatment for improving fibers content in GCII of both seasons and significantly equaled ($p \geq 0.05$) all other combination treatments in this respect, except $IP_{0\%} \times PD_{33}$, $IP_{20\%} \times PD_{22}$, and $IP_{40\%} \times PD_{33}$ in SI. The most effective combination ($p \leq 0.05$) between IP and PD for improving non-structural carbohydrates in both GCI and GCII of SI and SII was $IP_{0\%} \times PD_{33}$. However, the difference between $IP_{0\%} \times PD_{33}$ and each of $IP_{0\%} \times PD_{22}$ (for GCI of SI), $IP_{0\%} \times PD_{33}$ (for both GCI and GCII of SII) and all plant densities under $IP_{20\%}$ did not reach the level of significance ($p \geq 0.05$).

3.4 Forage and Protein Yields and Irrigation Water Use Efficiency

The interaction between IP and PD had significant influence ($p \leq 0.05$) on the aggregate protein yield and irrigation water-use efficiency (IWUE) in both seasons and aggregate dry forage yield only in SI (Fig. 3). The best aggregate protein yield for SI and SII was obtained under $IP_{20\%} \times PD_{33}$

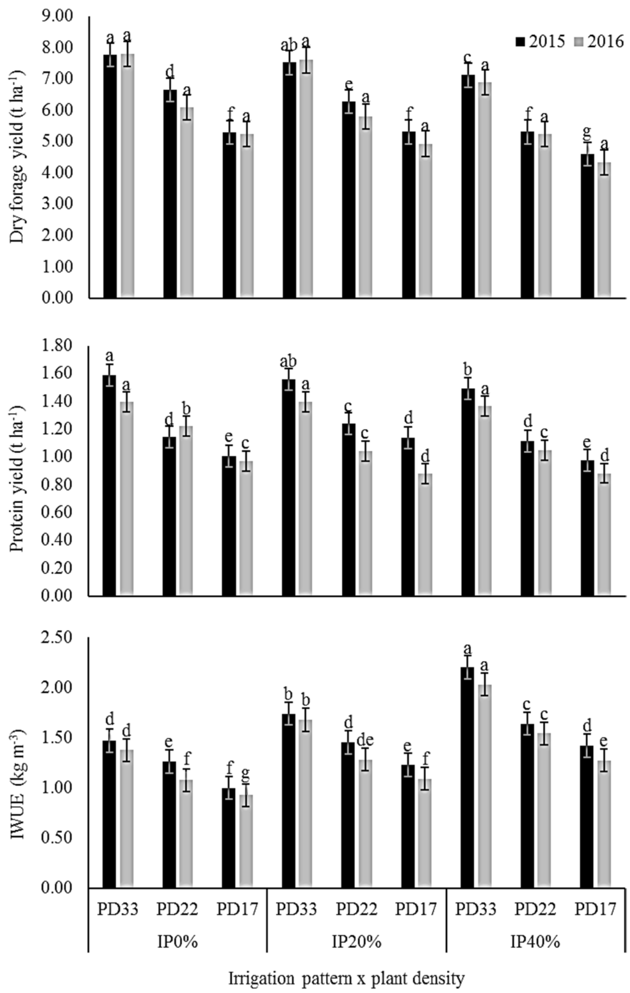

Fig. 3 Effect of irrigation pattern and planting density interaction on aggregate dry forage yield, aggregate protein yield and irrigation water use efficiency (IWUE) of clitoria in 2015 and 2016 seasons. IP_0%, IP20%, and IP40%: irrigation by 100, 80, and 60% of allowed soil water depletion, respectively. PD33, PD22, and PD17: planting density of 33, 22, and 17 plants m^{-2}, respectively. Means within the same bar of the same season followed by the same letter are not significantly different at $p \leq 0.05$ according to Duncan's test

interaction without significant difference ($p \geq 0.05$) with $IP_{0\%} \times PD_{33}$ or $IP_{40\%} \times PD_{33}$ interactions. The greatest aggregate dry forage yield was observed in SI under $IP_{0\%}$ or $IP_{20\%}$ combined with PD_{33} which did not differ significantly ($p \geq 0.05$).

3.5 The Significant Main Effects

It is interesting to note that some traits of clitoria did not significantly responded to the interaction between IP and PD. Thus, it could be presented the individual effect of IP and PD as shown in the supplementary Tables a and b. In this regard, all tested traits showed the highest values with well-watered conditions ($IP_{0\%}$) except ion leakage which recorded the maximum value under water-stressed conditions ($IP_{40\%}$) in both seasons. The differences between $IP_{0\%}$ and $IP_{20\%}$ were not significant for chlorophyll content, plant height, and leaf number plant^{-1} in the first season. On the other hand, PD_{33} was the potent practice for enhancing cell membrane stability index and plant height in the first season as well as leaf relative water content, ion leakage, plant height, and leaf area index in the second season. However, PD_{33} possessed the maximal values of chlorophyll content, ion leakage, leaf number plant^{-1}, and branch number plant^{-1} in the first season as well as dry weight plant^{-1} in the second season.

3.6 Regression Relationships

The functional relationship revealed that there is an increase in aggregate dry forage yield and a decrease in IWUE with increasing the amount of applied irrigation water in a quadratic function in SI and SII (Fig. 4). Regression equations are forecasting that the higher the applied irrigation water increases by one unit the higher the forage yield increases by 0.0022 and 0.0017 as well as IWUE decreases by 0.0003 and 0.0004, in SI and SII, respectively.

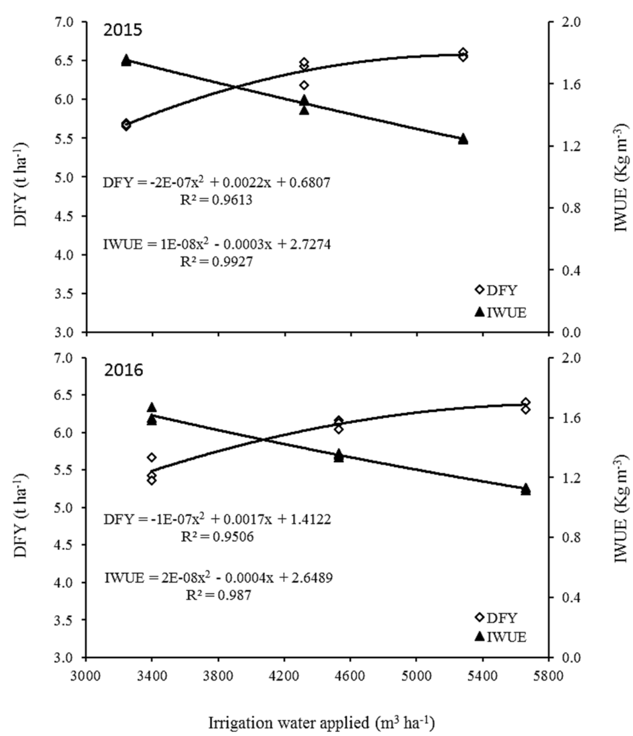

Fig. 4 Functional relationship between irrigation water applied and each of aggregate dry forage yield (DFY) and irrigation water use efficiency (IWUE) of clitoria in 2015 and 2016 seasons. **$p \leq 0.0$

4 Discussion

It is well known that plants exposed to any type of stress led to disturbance in their physiological status resulting in an unfavorable change in growth and development (Saudy and Mubarak 2015; Abd El-Mageed et al. 2021; Abd El-Mageed et al. 2022; Abou Tahoun et al. 2022). Therefore, several tools and tactics have been adopted to enhance the health status of crop plants (Saudy et al. 2021b; El-Metwally et al. 2022b; El-Metwally et al. 2022c; Saudy et al. 2022b; Shaaban et al. 2023). At the cellular scale, the deficit irrigation treatments cause a reduction in chlorophyll content in clitoria plants, which is partly related to the quick degradation of chlorophyll compared to its too slow construction under the reduced photosynthetic activity, which is likely correlating to decreasing activity of the RuBisCO enzyme, transpiration process, stomatal closure, and intercellular CO_2 concentration (El-Enany et al. 2014; Makhlouf et al. 2022). Moreover, decreasing leaf relative water content is one of the earliest markers of water reduction in plant tissue cells (Mahfouz et al. 2020). Studies conducted on alfalfa (Slama et al. 2011) and *Bituminaria bituminosa* L. (Martínez-Fernández et al. 2012) showed reduction in leaf relative water content under drought stress. The reduction in leaf relative water content under deficit water conditions was caused by water depleted from the soil by clitoria plants; thence, their roots are not able to recompense water consumed in the photosynthetic process (Sepanlo et al. 2014). The slight decline of leaf relative water content in clitoria could point out that this legume plant owns a drought escape mechanism to sustain a convenient water status in their tissues.

Under prolonged severe deficit water, reactive oxygen species (ROS) can be over-accumulated in the plant's cells causing oxidative damage to their biomolecules such as phospholipids, proteins, deoxyribonucleic acid, pigments, and other cellular compounds, hence damaging the cell membrane (Ramadan et al. 2023; Farooq et al. 2009). Premachandra et al. (1991) reported that the cell membrane stability index is a physiological sign vastly related to the plant's ability to drought tolerance. Accordingly, the cell membrane stability index decreased in plants of clitoria exposed to severe deficit water. A major mechanism to maintain tissue turgor under soil water deficit is lowering the cell osmotic potential by increasing the accumulation of some osmoprotectants including proline and other solutes in the cytoplasm and thus attract water into the cells and tissues. Other adaptive roles have been reported to proline such as scavenging ROS and therefore help in reducing oxidative stress and safeguard cell membrane integrity (Trovato et al. 2008), conservation of ion uptake, and water balance inside plant cells (Chiulele and Agenbag 2004; Saudy and El-Metwally 2019; Salem et al. 2022; El-Hashash et al. 2022; Saudy et al. 2023b).

The results also indicated that increasing soil water depletion through deficit irrigation from 20% to 40% further decreased almost all morpho-physiological attributes in both seasons. These declines of morphological attributes primarily due to the negative impacts of drought stress are emphasized by Nonami (1998) who reported that deficit irrigation stress results in a reduction in turgor pressure of cells by the hindrance of water outflow from the xylem to the adjoining elongating cells caused suppression and inhibition of the growth process. These results are in harmony with those reported by Testa et al. (2011), Abbas et al. (2017), Saudy et al. (2022c), and Saudy et al. (2022d). However, the leaf: stem ratio enhanced as water stress increased. Similar trends have been reported on alfalfa (Li and Su 2017).

Except for protein content, the other forage nutritive value indices, i.e., fiber and non-structural carbohydrates, decreased with increasing deficit water (Abbas et al. 2017). This result is mainly attributed to reducing dry matter buildup in plants of clitoria that underwent deficit water conditions as reported in soybean (Nielsen 2011). However, the raising protein content in clitoria plants exposed to deficit water treatment was expected due to the high leaf: stem ratio, which confirms the findings of Testa et al. (2011) who reported that protein content was strictly correlated (R^2= 0.76) to leaf: stem ratio in alfalfa.

The findings indicated that the dry forage yield of clitoria increased with the increase of irrigation water quantity. These findings are in line with those mentioned by Li and Su (2017) and Mahfouz et al. (2020) on alfalfa and forage clitoria. Nielsen (2011) highlighted that the increase in dry forage yield of forage soybean was mainly owing to greater plant height, leaf number plant^{-1}, branch number plant^{-1}, leaf area index, dry weight plant^{-1}, chlorophyll content, and absolute growth rate when irrigated by $IP_{0\%}$ level compared with stress ($IP_{20\%}$ and $IP_{40\%}$) conditions. Diniz et al. (2002) stated that clitoria has moderate drought tolerance, which is verified by our findings through the decrease of leaf relative water content and cell membrane stability index plus the increase of proline in their tissues. Thence, clitoria plants grown under $IP_{20\%}$ have occasionally succeeded in parallelizing $IP_{0\%}$ in terms of performance and productivity. Higher IWUE for clitoria plants grown under severe deficit ($IP_{40\%}$) compared to plants grown under moderate ($IP_{20\%}$) and well-watered ($IP_{0\%}$) as confirmed by Salgado et al. (2010) could be ascribed to the lower quantity of irrigation water applied compared to the dry biomass increase and accordingly greater IWUE (Wilson et al. 2012; El Sherbiny et al. 2022).

Our findings revealed also that with the increase of plant density from PD_{17} to PD_{33} significant increase in the plant height of clitoria was detected (Kumalasari et al. 2017). This probably is due to the high rate of stem elongation which is related to the intra-specific competition for pre-empting light, water, and nutrient supplies in addition to the shading effect in high plant density levels which promoted vertical growth of the plants (Craine and Dybzinski 2013). However, decreases were detected with the increase of plant density from PD_{17} to PD_{33} in terms of leaf number plant^{-1}, branch number plant^{-1}, dry weight plant^{-1}, and absolute growth rate in both seasons. Similar trends were previously observed in other forage crops by Kumalasari et al. (2017). These decreases could be explained by two integral interpretations. First, by reducing the plant spacing in high plant density, the plants displayed a stronger apical dominance in search of solar radiation, thus producing fewer lateral branches compared to those in wider plant spacing (Streck et al. 2014). Second, the early canopy closure for plants grown under narrow plant spacing reduces their ability to intercept solar radiation that reaches the leaves and reduces their chlorophyll content and weakening photosynthesis rate and hence undermining plant growth (Mattera et al. 2013). Worth mentioning that the thinner stems of plants grown with high plant density could provide faster field drying at harvest and may reduce the coarse stem sections by the animal at feeding (Hintz et al. 1992). Also, the findings of Mattera et al. (2013) and Mojaddam and Noori (2015) showed that clitoria plants grown under high planting density have higher leaf area index than those under low plant density. The higher leaf area index in closer plant spacings might be due to the increase in the number of leaves produced per unit area under this condition.

In general, intra-specific plant-to-plant competition is important environmental stress affecting the production of biomass yield as well as economic profitability (Ramanjaneyulu et al. 2018). Our results revealed that the leaf relative water content and cell membrane stability index decreased but ion leakage and proline increased with lower plant density, particularly in GCI. These findings might be owing to the higher soil evaporation compared to plant evapotranspiration under widening plant spacing within the ridge before the full establishment of plant canopy that shades the soil surface, depending on air temperature and relative humidity particular in the early stages of clitoria plant. Thus, early vigorous growth for plants might be an effective way for establishing canopy cover quicker to minimize soil water evaporation. On the other side, the leaf relative water content and cell membrane stability index increased while ion leakage and proline decreased with increasing plant density, especially in GCII of clitoria plant. This finding was supported by Suresh et al. (2013) on pigeon pea plant. These results are probably ascribed to improving soil and leaf water status resulting from the decreasing surface soil evaporation due to near complete coverage by plant canopy under narrow hill spacings with more uniform planting distribution and therefore increase dry matter content of the plant.

There is a clear increase in forage protein in both GCI and GCII under high plant density as previously reported in sainfoin (Stevović et al. 2012) and cowpea (Helmy et al. 2015). This result under our experiment is likely associated with increasing leaf: stem ratio. On the other hand, the increments of fibers and non-structural carbohydrates as plant density decreases might be ascribed to the vigorous growth of the root system with wider row spacings which enhances water and nutrient uptake that would result in a greater canopy leaf area development and greater light interception. Consequently, increasing in accumulate of dry matter components in the plant was achieved. These findings agree with those obtained by Ayub et al. (2011). The results of forage quality exhibit clear evidence for a strong relationship between adequate plant density interacting with soil water availability and its direct effect in obtaining a high nutritional value of forage clitoria. Concerning forage yield, our observations indicate that, despite the dry weight plant^{-1} for individual plants decreased as the plant density per unit area increased, the high number of plants per unit area compensated the reduction in weight per plant, increasing the dry forage yield per area when the plant density increased (Stanisavljević et al. 2012). As well, the higher IWUE obtained under high plant density may be ascribed to the enhancement of dry forage yield by the amount of irrigation water applied (Zhou et al. 2015).

Finally, there was a significant effect of irrigation water amount × plant density interaction on almost all studied parameters for both GCI and GCII of clitoria; the highest values of dry forage yield were observed with $IP_{20\%} \times PD_{33}$ and $IP_{0\%} \times PD_{33}$ interactions. The improvement of soil water status resulting from the decreasing of soil evaporation due to near complete coverage by plant canopy under narrow high density might partly be the reason for enabling plants to tolerate moderate drought stress ($IP_{20\%}$), thereby maintaining high yield despite the water stress as appeared in this study. Thus, the determination of the appropriate plant density may be a practicable practice to mitigate the negative effects of drought associated with limited irrigation water in arid agroecosystems.

5 Conclusions

Findings proved that clitoria is a moderate drought tolerance plant, since decrease in leaf relative water content and cell membrane stability index and increase in proline were discovered under deficit water. Exposing clitoria plants to drought stress through deficit irrigation resulted in a decrease in their physio-biochemical and morphological attributes. It also decreases forage nutritive value (i.e., fiber and non-structural carbohydrates), and yield of aggregate dry forage, however, exhibited increases in leaf: stem ratio, ion leakage, proline, protein content, and irrigation water use efficiency, while planting density through changing hill spacing seemed to fluctuate factor compared to deficit water regimes. Thus, plant density of 33 plant per square meter maximized the productivity of clitoria with satisfying nutritive value indices and improved water utilization. Therefore, adjusting the plant density of clitoria at 33 plant per square meter (330000 plant per hectare) is the appropriate agricultural pattern for obtaining high productivity under deficit irrigation (irrigation by 80% of full water amount) while saving water by 20% in arid agroecosystem conditions.

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Authors Contribution Ahmed Shaaban, Hamdy Mahfouz, Ekram Ali Megawer, and Hani Saber Saudy conceived and designed the experiment, handled the experiment and measured physiological indicators, analyzed the data, and wrote the paper. All authors read and approved the final manuscript

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Declarations

Conflicts of Interest The authors declare no competing interests.

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