Effects of Regulated Deficit Irrigation on Yield and Quality of *Isatis indigotica* in a Cold and Arid Environment

Yucai Wang 1, Hengjia Zhang 1,*, Zhongsheng He 2, Fuqiang Li 1, Zeyi Wang 1, Chenli Zhou 1, Yi Han 1 and Lian Lei 3

1 College of Water Resources and Hydropower Engineering, Gansu Agricultural University, Lanzhou 730070, China; wangyucail18@163.com (Y.W.); gsnzyd1@126.com (F.L.); peterwangzy@126.com (Z.W.); zhouchenli2021@126.com (C.Z.); cehanyi@163.com (Y.H.)

2 Xinjiang Bazhou Academy of Agricultural Sciences, Korla 841000, China; hzs096@163.com

3 Yimin Irrigation Experimental Station, Zhangye 734500, China; leilian1122022@163.com

*Correspondence: zhangjh@gsau.edu.cn; Tel.: +86-182-1971-2357*

Abstract: Although regulated deficit irrigation may improve crop yields, little research has been conducted on the effects of water deficits on *Isatis indigotica*, a popular herbal medicine. Field experiments were conducted in 2016 and 2017 to study the effects of regulated deficit irrigation on the net photosynthetic rate, yield, water use efficiency (WUE), and quality of *I. indigotica* in northwest China. Plants at the vegetative and fleshy root growth stages were subjected to mild, moderate, and severe water deficits, and their photosynthetic physiological indexes, yield, and WUE were measured. Moderate and severe deficits, but not a mild deficit, significantly decreased the net photosynthetic rate and dry matter accumulation. The yield and WUE under mild deficit were markedly higher, reaching 8239.56 kg·ha⁻¹ and 8390.80 kg·ha⁻², respectively, in the vegetative stage and 24.11 kg·ha⁻²·mm⁻¹ and 23.62 kg·ha⁻²·mm⁻¹, respectively, in the fleshy root growth stage, while severe deficits significantly reduced yield and WUE. Mild and moderate deficits increased the content of (R,S)-goitrin, indirubin, and indigo, improving root quality, but severe deficits decreased these compounds. Therefore, a mild water deficit in the vegetative and fleshy root growth stages is optimal and can reduce water consumption and improve *I. indigotica* quality and WUE without reducing yield.

Keywords: water deficit; dry matter; yield; quality; water use efficiency; *Isatis indigotica*

1. Introduction

*Isatis indigotica* is one of the most commonly used Chinese herbal medicines. In recent years, it has been found that *Isatis* root and *Isatis* leaf are broad-spectrum plant antibiotics that can be clinically used to treat various inflammations of the respiratory and circulatory systems [1]. *I. indigotica* has been widely introduced in China due to its strong adaptability to various climate, soil, and environmental conditions, with Hebei, Shaanxi, Gansu, and Jiangsu becoming its main production areas. However, in the Hexi Corridor, due to the shortage of water resources and unreasonable irrigation methods used, the crop yield and water use efficiency (WUE) are low, which largely restricts the sustainable development of the *I. indigotica* industry. Changes in irrigation and planting methods are needed to improve the yield, WUE, and quality of *I. indigotica*. Relevant international studies have shown that the technology of regulated deficit irrigation can be applied to tomatoes, lemon trees, various citrus fruits, onions, and other crops [2–5]. Many reports on the application of regulated deficit irrigation in practical production [6–10] have studied the response of yield and quality of different crops, including tomatoes, alfalfa, watermelon, cucumbers, and wine grapes, to regulated deficit irrigation. Studies have shown that regulated drip irrigation results in improved water-saving and
yield-increasing performance, consequently improving crop quality. Some scholars have studied the influence of water deficits on the growth and yield of *I. indigotica* and found that, when the maximum water-holding capacity was 45–70%, the yield and quality were the best [11].

Limited irrigation and drip irrigation methods have effectively increased the yield and quality of crops such as onions [12], tomatoes [13], melons [14], and squash [15]. It has been experimentally determined that controlled deficit irrigation reduces water use by 5% compared with constant deficit irrigation and improves productivity by 4% for orange trees [16]. A previous study demonstrated that the irrigation of sugar beet with the drip irrigation method at a 75% level had significant benefits in terms of saved irrigation water and high WUE; this showed that, under the condition of limited water supply, under-irrigation had obvious advantages [17]. Some scholars have studied the effect of drip irrigation under different membranes on sugar beet production in Xinjiang. When the irrigation amount was 60 mm and the frequency was 11 d, the sugar yields of sugar beet increased by 10.8% and 6.5%, respectively [18].

At present, research on *I. indigotica* in China and abroad mainly focuses on its chemical composition, pharmacological activity, plant characteristics, preparation, and quality evaluation [19]. Studies on the effects of regulated water deficits on *I. indigotica* in China and abroad conducted water-regulation experiments for the whole growth stage of *I. indigotica*, while neglecting the growth status, yield, and quality at different growth stages. In addition, there are few studies on the effect of water deficits on the growth and yield of *I. indigotica* under drip irrigation under mulch [11]. The application of regulated deficit irrigation has achieved good results in different crops [2], and it is assumed that a water deficit under drip irrigation will affect the yield and quality of *I. indigotica*. The contents of indigo, indirubin, and (R, S)-goitrin indicate the quality of *I. indigotica*. Through a field experiment on *I. indigotica* in the city of Zhangye, Minle County, Hexi Corridor, the effects of a regulated water deficit on the growth dynamics, yield, and quality at different growth stages under drip irrigation with mulch were investigated. The method of drip irrigation under mulch was further explored, providing a theoretical basis for the high-yield cultivation of *I. indigotica* in arid areas of northwest China.

2. Materials and Methods

2.1. Experimental Site

The experiments were carried out at Yimin Irrigation Pilot Station (100°43′ E, 38°39′ N) in the middle reaches of the Flood River Irrigation District, Minle County, Gansu Province, from May to October in 2016 and 2017. The experimental zone has a continental desert steppe climate, with a dry climate, abundant heat, abundant light energy, and little rain; the altitude is about 1970 m; the annual average temperature is 6.0 °C; ≥10 °C the cumulative temperature is 3500 °C; ≥10 °C the effective cumulative temperature is 2985 °C; the extreme maximum temperature is 37.8 °C; the extreme minimum temperature is −33.3 °C; the average sunshine hours are 3000 h per year, and the frost-free period is 125 days. The average annual precipitation in this area is 215 mm. The contradiction between water supply and the water demand for crops in this area is prominent, and drought occurs from time to time. The soil is light loam with a pH value of 7.22; the field water holding capacity of tillage layer soil is 24%; the soil bulk density is 1.4 g·cm⁻³; the groundwater level is low, and the area does not exhibit salinization or alkalinization.

2.2. Test Materials and Cultivation Methods

The seeds of *I. indigotica* were independently planted in the department of Chinese herbal medicine at Gansu Agricultural University, where the seed purity was 96% and the weight per 1000 seeds was 9.873 g, while the germination capacity was 87.6%. Seeds were sown on May 3, and crops were harvested on October 13. The seeding rate was 30 kg per hectare (kg·ha⁻¹), and the cropping density was 700,350 plants per hectare (ha). The
experimental land was plowed mechanically to a depth of 30 cm, and weeds were removed mechanically before planting. In addition, 210, 340, and 270 kg·ha⁻² urea (N content 46%), superphosphate (P₂O₅ content 12%, S content 10%, and Ca content 16%), and source potassium (K₂O content 60%), respectively, were added as basic fertilizers. Before *L. indigotica* was planted, three drip irrigation belts were manually laid in each plot with a distance of 1 m between them, a distance of 30 cm between drip emitters, and an average flow rate of 2.5 L/h. A branch control valve was installed in each plot to control the irrigation quantity; a pressure gauge and water meter were located at the drip irrigation hub, and the working pressure of the system was 0.1 MPa. After the drip irrigation system was installed, the soil was covered with colorless plastic film with a width of 120 cm. Each experimental plot was separated by a film with a width of 60 cm to prevent water seepage underground. The experimental research and field studies on plants, including the collection of plant material, were conducted in accordance with the irrigation experiment standard (SL 13–2015) issued by the Water Industry Standards of China. The irrigation test system for different crops was developed by the China water conservancy industry. The permission for the collection of *L. indigotica* was approved by the Yimin Irrigation Experimental Station and Flood River Administration Office, Minle County, China.

2.3. Experiment Design

The growth of *L. indigotica* was divided into four stages according to their growth characteristics: the seedling stage (3 May–7 June), vegetative stage (8 June–18 July), fleshy root growth stage (19 July–28 August), and fleshy root maturity (29 August–13 October). The field water-holding capacity refers to the relatively stable soil water content that can be maintained in the soil profile after sufficient irrigation or precipitation on land with deep groundwater and good drainage; in addition, it is the upper limit of soil water available to most plants. The field water holdup was measured by the ring knife method in the laboratory. Four deficit levels were classified for soil moisture: adequate irrigation (soil water content was 75–85% of field water capacity), mild water deficit (soil water content was 65–75% of field water capacity), moderate water deficit (soil water content was 55–65% of field water capacity), and severe water deficit (soil water content was 45–55% of field water capacity). There were 10 water control treatments, of which CK was the control treatment. Each treatment was repeated three times for a total of 30 plots. The area of each plot was 36 m² (9 m × 4 m), and a random block design was adopted. The effective experimental planting area was 1080 m². The method of irrigation used was drip irrigation under mulch. Soil moisture was controlled within the designated range. When the soil moisture of the planned wet layer was reduced to the lower design limit, irrigation was applied to reach the upper limit. The re-watering was the process of leaving crops under water stress and then returning to normal water supply. The specific experimental design is shown in Table 1.

**Table 1.** Water-deficit irrigation treatments at different levels and different growth stages.

| Treatments | Seedling Stage | Vegetative Stage | Fleshy Root Growth Stage | Fleshy Root Maturity |
|------------|----------------|------------------|-------------------------|---------------------|
| CK         | 75–85%         | 75–85%           | 75–85%                  | 75–85%              |
| WD1        | 75–85%         | 65–75%           | 75–85%                  | 75–85%              |
| WD2        | 75–85%         | 55–65%           | 75–85%                  | 75–85%              |
| WD3        | 75–85%         | 45–55%           | 75–85%                  | 75–85%              |
| WD4        | 75–85%         | 65–75%           | 65–75%                  | 75–85%              |
| WD5        | 75–85%         | 65–75%           | 55–65%                  | 75–85%              |
| WD6        | 75–85%         | 55–65%           | 65–75%                  | 75–85%              |
| WD7        | 75–85%         | 55–65%           | 55–65%                  | 75–85%              |
| WD8        | 75–85%         | 45–55%           | 65–75%                  | 75–85%              |
| WD9        | 75–85%         | 45–55%           | 55–65%                  | 75–85%              |

WD1: mild water deficit in the vegetative stage, WD2: moderate water deficit in the vegetative stage, WD3: severe water deficit in the vegetative stage, WD4: mild water deficit in the vegetative stage.
stage and fleshy root growth stage, WD5: mild water deficit in the vegetative stage and moderate water deficit in the fleshy root growth stage, WD6: moderate water deficit in the vegetative stage and mild water deficit in the fleshy root growth stage, WD7: moderate water deficit in the vegetative and fleshy root growth stage, WD8: severe water deficit in the vegetative and mild water deficit in the fleshy root growth stage, WD9: severe water deficit in the vegetative and moderate water deficit in the fleshy root growth stage, CK: normal water supply in all growth stages.

2.4. Indicators and Methods for Measurement

2.4.1. Soil Moisture Content

During the growth period, the SMC was measured using the traditional drying method every 7–10 days, randomly at the midpoint of the line of two *I. indigotica* plants, with 3 measuring points in each plot. This was done using soil drills to sample six layers; one sample was taken in the first 10 cm of the soil. Then, samples were taken at intervals of 20 cm, from 20–100 cm in depth. Because the root system of the *I. indigotica* is primarily distributed in the 50-cm soil layer, the average value of water content in the 60-cm soil layer was used as the basis for irrigation.

The formulas for calculating the irrigation amount for the *I. indigotica* are

\[ \theta = \left( m_a - m_b \right) / m_b, \text{ and} \]

\[ M = 10\gamma H_p (\theta_i - \theta_j) \]

where \( \theta \) is the soil mass water content (%); \( m_a \) and \( m_b \) are the weights of fresh soil and dry soil, respectively, \( g \); \( M \) is the amount of irrigation water (mm); \( \gamma \) is the volume density of the planned wet layer (g cm\(^{-3}\)); \( H_p \) is the depth of the planned wet layer (60 cm); \( \theta_i \) is the design control upper limit moisture content (field water capacity multiplied by upper limit of design control relative moisture content,%); \( \theta_j \) is the mass moisture content of the soil before irrigation (%), and \( P \) is the design wet ratio of the drip irrigation (65%).

2.4.2. Water Consumption

The stage water consumption was calculated using the water-balance method:

\[ ET_{1-2} = 10\sum_{i=1}^{n} \gamma_i H_i (W_{i1} - W_{i2}) + M + P + K - C \]

where \( ET_{1-2} \) is the stage water consumption of the indigowoad root (mm); \( i \) and \( n \) are the soil layer number and total number, respectively; \( \gamma_i \) is the \( i \)-th layer soil bulk density; \( H_i \) is the \( i \)-th layer soil layer thickness (cm); \( W_{i1} \) and \( W_{i2} \) are the moisture content of the \( i \)-th soil at the beginning and end of a certain period (%); and \( M, P, K, \) and \( C \) are the amount of irrigation, precipitation, supplement, and drainage of deep soil water, respectively, during this period (mm). The depth of the groundwater in the test region was greater than 20 m, so there was no need to consider deep water supplementation, and \( K \) was 0. The test region was an arid area, and drip irrigation failed to make the SMC reach the saturation value. No leakage occurred. Hence, \( C \) was taken to be 0.

2.4.3. Dry Matter and Yield

Four measurements were made from the emergence of *I. indigotica* to the end of each growth period. Five plants with the same growth status were selected from each plot for each measurement. After being dug out, the root soil was gently brushed off with a fine brush. The leaves, stems, and roots were cut from the plant, washed and dried indoors,
marked with paper bags, and packed separately. The temperature was fixed at 105 °C for 30 min; then the temperature was adjusted to 80 °C, and the samples were dried to a constant weight. The above-ground and underground dry matter mass were measured by electronic weighing to a precision of 0.001 g. After the fleshy roots were mature, 20 seedlings were harvested and brought back to the laboratory for washing and drying and were used to calculate the yield per hectare.

2.4.4. Leaf Gas Exchange

The net photosynthetic rate and stomatal conductance of leaves were measured at 9:00–11:00 a.m. on the seventh day after regulated water-deficit treatment with an LI-6400 portable photosynthesis meter (LI-COR, Lincoln, NE, USA) under a photon intensity of 1000 μmol·m⁻²·s⁻¹. The middle part of the third functional leaf from the inside to the outside was selected for measurement and labeled. Five plants were measured in each plot.

2.4.5. WUE and Irrigation WUE (IWUE)

The calculation formulas for WUE and IWUE are as follows:

\[
WUE = \frac{Y}{ET_{a}}, \tag{4}
\]

\[
IWUE = \frac{Y}{I}, \tag{5}
\]

where WUE is the WUE in kg·ha⁻²·mm⁻¹; IWUE is the IWUE in kg·ha⁻²·mm⁻¹; Y is the yield per unit area of *I. indigotica* (kg·ha⁻²·mm⁻¹); ETₐ is water consumption (mm), and I is the irrigation water amount (mm) during the whole growth period of *I. indigotica*.

2.4.6. Quality Measurement

For sample preparation, samples of *I. indigotica* were collected, washed, wetted, and sliced after impurities were removed. They were then dried to constant weight at 60 °C in an oven, crushed, and sifted through a 40-mesh sieve. One gram of *I. indigotica* root powder was weighed and placed in a conical flask. Then, 50 mL of deionized water was added, weighed, and recorded. After 50 min of sound extraction (power 500 W, frequency 40 kHz), the samples were taken out, weighed after cooling, and water was used to make up the lost weight. The samples were well shaken and then filtered with a 0.45-μm membrane. The sample solution was then obtained. Six sample solutions were prepared simultaneously for each sample.

High-performance liquid chromatography (LC-10ATVP, Shimadzu Corporation, Kyoto, Japan) conditions were as follows. An SPD-10AVP (UV-VIS) detector and an Agilent Zorbax SB-C18 chromatographic column (100 mm × 4.6 mm, 3.5 μm) were used; the mobile phase was methanol–0.1% formic acid solution; the flow rate was 1.0 mL·min⁻¹, equipped with an automatic sampler, and the injection volume was 20 μL; the detection wavelength was 280 nm, and the column temperature was 25 °C. Indigo, indirubin, and (R,S)-goitrin standards were provided by the National Institutes for Food and Drug Control.

For the preparation of the reference solution, 1.0 mg of indigo standard, indirubin standard, and (R,S)-goitrin standard were weighed and placed in a beaker, dissolved with an appropriate amount of ethanol, diluted to 100 mL with ethanol, and shaken well to obtain sample solutions. Then, 0.5 mL of the standby solution was poured into a 10-mL measuring cylinder, and the calibration solution was diluted with ethanol and shaken well to obtain a 0.5 μg/mL reference solution.

To measure the sample content, 20 mL of each of the label solution and sample solution were precisely measured and absorbed, with samples injected six times.

2.5. Data Analysis

The average value of each treatment was calculated in 2016 and 2017. A chromatogram was recorded, and the peak area was calculated. The contents of
indirubin, indigo, and (R, S)-goitrin were calculated according to the external standard method. The experiment data were analyzed using a one-way ANOVA using the SPSS software package (Version 20.0, Stanford University). Excel 2010 was used to calculate the measured data. Duncan’s multiple comparison method in SPSS 19.0 software was used to compare the significance of the differences between the data processed. GraphPad Prism 5.01 was used to draw the graph. The data in each table are the average values of three replicates.

3. Results
3.1. Temperature and Precipitation Dynamics in the Whole Plant Growth Period

Although the average daily temperature fluctuated within a certain range during the whole growth period, the fluctuation was similar to the local temperature variation in the past five years (Figure 1). In 2016, the lowest average daily temperature was 12.8 °C, which appeared at the beginning of the seedling stage, and the highest average daily temperature was 27.6 °C, which appeared at the fleshy root growth stage. The average daily temperatures during the whole growth period in 2017 were 3.8 °C at the beginning of the seedling stage and 2.5 °C at the later maturity of fleshy roots. The maximum average daily temperature was 29.8 °C at the fleshy root growth stage. In 2016, the total precipitation in the whole growth period was 185.8 mm and was mainly concentrated in July and August. The precipitation was mainly concentrated in the fleshy root growth stage and at maturity, during which the amounts reached 72.8 mm and 60.5 mm, respectively, accounting for 39.2% and 32.6% of the total precipitation of the whole growth period, respectively. The precipitation in 2017 was 196.5 mm in the whole growth period, and the precipitation was evenly distributed in each growth stage. The precipitation in the late vegetative period and the fleshy root growth period in 2017 was less than that in 2016.

Figure 1. Average temperature and precipitation changes during different growth stages. (A) The experimental data in 2016. (B) The experimental data in 2017.
3.2. Water Consumption

During the entire growth period, the water consumption of each water-deficit treatment was affected by drip irrigation under plastic film (Table 2). CK had the highest water consumption during the whole growth period (374.04 mm in 2016 and 381.75 mm in 2017), and the other treatments had significantly lower water consumption during the whole growth period (p < 0.05). With the gradual aggravation of the deficit degree, the water consumption in the whole growth period of other treatments also showed a trend of gradual decline. Therefore, the degree of water-regulation deficit affected the water consumption of *I. indigotica* during all growth stages, and with the increase of the degree of regulated deficit irrigation, the decrease in water consumption during the whole growth period was more obvious.

Table 2. *I. indigotica* water consumption at different growth stages.

| Year | Treatment | Seedling Stage | Vegetative Stage | Fleshy Root Growth Stage | Fleshy Root Maturity | Whole Growth Period |
|------|-----------|----------------|------------------|--------------------------|---------------------|---------------------|
| 2016 | CK        | 33.56 a        | 138.09 a         | 125.67 a                 | 76.72 a             | 374.04 a            |
|      | WD1       | 35.08 a        | 128.77 b         | 126.55 a                 | 52.88 d             | 343.28 bc           |
|      | WD2       | 34.86 a        | 126.65 bc        | 118.52 b                 | 73.02 ab            | 353.05 b            |
|      | WD3       | 35.21 a        | 110.76 f         | 117.65 b                 | 72.30 ab            | 353.92 c            |
|      | WD4       | 34.13 a        | 125.65 bc        | 118.56 b                 | 62.51 c             | 340.85 c            |
|      | WD5       | 32.45 a        | 120.01 cde       | 117.43 b                 | 76.17 a             | 346.06 bc           |
|      | WD6       | 33.78 a        | 117.36 def       | 120.67 b                 | 66.57 bc            | 338.38 c            |
|      | WD7       | 31.87 a        | 122.56 bcd       | 119.54 b                 | 64.59 c             | 338.56 c            |
|      | WD8       | 31.11 a        | 115.33 def       | 109.21 c                 | 60.38 c             | 316.03 d            |
|      | WD9       | 32.17 a        | 113.65 ef        | 105.21 c                 | 64.24 c             | 315.27 d            |

| 2017 | CK        | 35.55 a        | 140.89 a         | 128.48 a                 | 76.83 ab            | 381.75 a            |
|      | WD1       | 35.35 a        | 136.73 b         | 125.86 a                 | 57.31 d             | 355.25 cd           |
|      | WD2       | 35.46 a        | 128.57 c         | 127.85 a                 | 74.18 ab            | 366.06 b            |
|      | WD3       | 33.55 ab       | 113.52 f         | 122.35 b                 | 74.20 ab            | 343.62 f            |
|      | WD4       | 32.77 ab       | 126.87 c         | 125.65 a                 | 68.64 abc           | 353.93 cde          |
|      | WD5       | 33.58 ab       | 125.74 c         | 120.85 b                 | 77.48 a             | 357.65 c            |
|      | WD6       | 31.75 b        | 120.78 d         | 118.82 b                 | 77.31 a             | 348.66 def          |
|      | WD7       | 32.06 b        | 119.65 de        | 121.73 b                 | 73.91 ab            | 347.35 ef           |
|      | WD8       | 32.98 ab       | 117.37 e         | 114.22 c                 | 64.45 cd            | 329.02 g            |
|      | WD9       | 32.75 ab       | 118.87 de        | 108.36 d                 | 67.80 bc            | 327.78 g            |

Note: Values are the means of three replicates for each treatment. Different lowercase letters within a column indicate a significant difference among treatments at p < 0.05. WD1: mild water deficit in the vegetative stage, WD2: moderate water deficit in the vegetative stage, WD3: severe water deficit in the vegetative stage, WD4: mild water deficit in the vegetative stage and fleshy root growth stage, WD5: mild water deficit in the vegetative stage and moderate water deficit in the fleshy root growth stage, WD6: moderate water deficit in the vegetative stage and moderate water deficit in the fleshy root growth stage, WD7: moderate water deficit in the vegetative and fleshy root growth stage, WD8: severe water deficit in the vegetative and mild water deficit in the fleshy root growth stage, WD9: severe water deficit in the vegetative and moderate water deficit in the fleshy root growth stage, CK: normal water supply in all growth stages.

3.3. Leaf Gas Exchange

There was no significant change in the net photosynthetic rate at the seedling stage and fleshy root maturity stage, but water deficits at the vegetative stage and fleshy root growth stage had more significant effects on the net photosynthetic rate (Figure 2). In
2016, the net photosynthetic rate of all treatments was lower than that of the control group at the vegetative stage \((p > 0.05)\). Among them, the net photosynthetic rates of WD3 and WD9 decreased by 17.1% and 19.1%, respectively, compared with CK \((p < 0.05)\). The compensatory effect occurred in the WD1 and WD4 treatments after the samples were re-watered at the fleshy root growth stage. The net photosynthetic rates in WD1 and WD4 reached 19.62 \(\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}\) and 19.84 \(\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}\), respectively, exceeding that of CK, but that of WD3 only reached 16.05 \(\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}\), which was lower than that of CK. The net photosynthetic rates of WD5 and WD7 under a moderate water deficit decreased at the fleshy root growth stage but recovered after the samples were re-watered to rates that were just lower than that of CK, reaching 3.8% and 4.7%, respectively. In 2017, the net photosynthetic rates of WD1 and WD4 increased by 5.5% and 1.8%, respectively, compared with CK at the vegetative stage; other treatments were reduced from 2.2% to 15.6%. The net photosynthetic rate of WD1 increased by 8.5% compared with CK at the fleshy root growth stage; other treatments were reduced from 0.5% to 19.4%.

Figure 2. Net photosynthetic rates under various water treatments across the 2016 and 2017 cropping seasons. Different lowercase letters within a column indicate a significant difference among treatments at \(p < 0.05\). (A) The experimental data in 2016. (B) The experimental data in 2017. WD1: mild water deficit in the vegetative stage, WD2: moderate water deficit in the vegetative stage, WD3: severe water deficit in the vegetative stage, WD4: mild water deficit in the vegetative stage and fleshy root growth stage, WD5: mild water deficit in the vegetative stage and moderate water deficit in the fleshy root growth stage, WD6: moderate water deficit in the vegetative stage.
and mild water deficit in the fleshy root growth stage, WD7: moderate water deficit in the vegetative and fleshy root growth stage, WD8: severe water deficit in the vegetative and mild water deficit in the fleshy root growth stage, WD9: severe water deficit in the vegetative and moderate water deficit in the fleshy root growth stage, CK: normal water supply in all growth stages.

These findings indicate that, although the net photosynthetic rate is affected by water deficit at the vegetative stage and fleshy root growth stage, the net photosynthetic rate recovers after I. indigotica is re-watered at the fleshy root growth stage and fleshy root maturity, showing a greater water-compensation effect.

As can be seen from Figure 3, the stomatal conductance of I. indigotica leaves at all growth stages decreased due to water deficit, and the higher the degree of water deficit, the lower the stomatal conductance. However, the WD1 and WD4 treatments were exceptions to this trend. In 2016, the stomatal conductance under a mild water deficit in treatments WD1 and WD4 was slightly higher than that of CK in the vegetative growth stage, but there was no significant difference between WD1 and WD4 (p > 0.05). Compared with CK, WD3, WD8, and WD9 significantly decreased by 20.0%, 20.1%, and 25.7%, respectively (p > 0.05). The change in 2017 was similar to that in 2016 and decreased significantly with the intensification of the water deficit. Moderate or severe water deficit in the vegetative and fleshy root stages of I. indigotica resulted in a decrease in stomatal opening.

Figure 3. Stomatal conductance under various water treatments across the 2016 and 2017 cropping seasons. Different lowercase letters within a column indicate a significant difference among
treatments at \( p < 0.05 \). (A) The experimental data in 2016. (B) The experimental data in 2017. WD1: mild water deficit in the vegetative stage, WD2: moderate water deficit in the vegetative stage, WD3: severe water deficit in the vegetative stage, WD4: mild water deficit in the vegetative stage and fleshy root growth stage, WD5: mild water deficit in the vegetative stage and moderate water deficit in the fleshy root growth stage, WD6: moderate water deficit in the vegetative stage and mild water deficit in the fleshy root growth stage, WD7: moderate water deficit in the vegetative and fleshy root growth stage, WD8: severe water deficit in the vegetative and mild water deficit in the fleshy root growth stage, WD9: severe water deficit in the vegetative and moderate water deficit in the fleshy root growth stage, CK: normal water supply in all growth stages.

3.4. Dry Matter Accumulation

The dry matter accumulation in different growth stages of *I. indigotica* under drip irrigation under mulch is shown in Figure 4. The dry matter accumulation process of *I. indigotica* showed an “S”-shaped tendency during the whole growth period. The dry matter accumulation was slow from the seedling to the vegetative stage. From the vegetative to the fleshy root growth stage, the plants grew vigorously, and dry matter accumulation changed significantly from the fleshy root growth stage to fleshy root maturity. However, dry matter accumulation was slow at fleshy root maturity. In 2016, the highest dry matter accumulation per plant under WD1 was 7.38 g/plant in the vegetative period, and there was no significant difference between WD1 and CK (\( p > 0.05 \)); meanwhile, the other treatments were lower than CK. The dry matter accumulation under the WD3, WD7, WD8, and WD9 treatments was significantly lower than that in CK, and the reduction ranged from 12.8% to 17.6%. In the fleshy root growth period, there was no significant difference between WD1, WD2, WD4, and CK, but other treatments were inferior to CK in terms of dry matter accumulation. The WD1 and WD4 treatments significantly increased by 8.1% and 6.1%, respectively, compared with CK at fleshy root maturity, and the dry matter accumulation under moderate and severe water-deficit treatments was significantly lower than that of CK, with a decrease ranging from 12.5% to 31.1%. In 2017, there was no significant difference between mild and moderate water-deficit treatments and CK in the vegetative period. With severe water deficit, WD3, WD8, and WD9 significantly decreased by 18.3%, 23.1%, and 24.4%, respectively, compared to CK; WD1, WD2, and WD4 were not significantly different compared to CK in the fleshy root growth stage in terms of dry matter accumulation, while the other treatments significantly decreased compared to CK; WD1 increased by 4.0% compared to CK at fleshy root maturity, but there was no significant difference.
The dry matter accumulation processes of *I. indigotica* in 2016 and 2017 were similar. There was no significant difference in dry matter accumulation during the growth stage due to the lack of a water-deficit treatment at the seedling stage (*p* > 0.05). However, the dry matter accumulations in the vegetative stage and fleshy root growth stage under water-deficit treatment were significantly different. There were significant differences in the growth stage (*p* < 0.05) because the effect of water on the physiological growth of crops occurred in the fleshy root maturity stage. The dry matter accumulation of *I. indigotica* showed an "S"-shaped tendency over time during the whole growth period, but a severe water deficit significantly reduced the dry matter accumulation, while a timely and appropriate mild water deficit did not significantly affect the dry matter accumulation and was conducive to improving the WUE of crops.
3.5. Yield and WUE

In 2016, the yield of *I. indigotica* under CK was the highest under full irrigation (up to 8315.58 kg·ha⁻²) in the whole growth period, and the yield under other water-deficit treatments, except for WD1 and WD4, was reduced (Table 3). WD3, WD7, WD8, and WD9, which were treated with moderate and severe water deficits, showed a significant difference compared with CK (p < 0.05) and decreased by 17.1%, 16.2%, 36.1%, and 37.1%, respectively.

Table 3. Effects of different water deficits on the yield and water-use efficiency of *Isatis indigotica*.

| Year | Treatment | Precipitation (mm) | Total Water Consumption (mm) | Yield (kg·hm⁻²) | IWUE (kg·hm⁻²·m⁻¹) | WUE (kg·hm⁻²·mm⁻¹) |
|------|-----------|--------------------|-----------------------------|-----------------|----------------------|---------------------|
| 2016 | CK        | 185.8              | 374.04                      | 8315.58 a       | 50.94 b              | 22.23 b             |
|      | WD1       | 185.8              | 343.28                      | 8239.56 a       | 54.04 a              | 24.01 a             |
|      | WD2       | 185.8              | 353.05                      | 7219.67 b       | 49.03 c              | 20.45 d             |
|      | WD3       | 185.8              | 335.92                      | 6894.60 d       | 51.03 b              | 20.52 d             |
|      | WD4       | 185.8              | 340.85                      | 8215.52 a       | 54.67 a              | 24.11 a             |
|      | WD5       | 185.8              | 346.06                      | 7164.91 bc      | 49.32 c              | 20.70 cd            |
|      | WD6       | 185.8              | 338.38                      | 7083.69 c       | 49.68 c              | 20.93 c             |
|      | WD7       | 185.8              | 338.56                      | 6965.85 d       | 50.57 b              | 20.57 d             |
|      | WD8       | 185.8              | 316.03                      | 5311.57 e       | 46.10 d              | 16.81 e             |
|      | WD9       | 185.8              | 315.27                      | 5228.54 e       | 46.48 d              | 16.58 e             |
| 2017 | CK        | 196.5              | 381.75                      | 8322.25 a       | 50.36 bc             | 21.80 b             |
|      | WD1       | 196.5              | 355.25                      | 8390.80 a       | 54.57 a              | 23.62 a             |
|      | WD2       | 196.5              | 366.06                      | 7462.24 b       | 49.89 c              | 20.39 c             |
|      | WD3       | 196.5              | 343.62                      | 6800.36 e       | 51.47 b              | 19.79 d             |
|      | WD4       | 196.5              | 353.93                      | 8235.32 a       | 54.03 a              | 23.27 a             |
|      | WD5       | 196.5              | 357.65                      | 7051.11 c       | 48.25 d              | 19.72 d             |
|      | WD6       | 196.5              | 348.66                      | 6981.71 cd      | 49.11 cd             | 20.02 cd            |
|      | WD7       | 196.5              | 347.35                      | 6819.79 de      | 50.20 bc             | 19.63 d             |
|      | WD8       | 196.5              | 329.02                      | 5686.71 f       | 48.39 d              | 17.28 e             |
|      | WD9       | 196.5              | 327.78                      | 5539.79 f       | 48.48 d              | 16.90 e             |

Note: Different lowercase letters within each column indicate significant differences at p < 0.05. WUE: water use efficiency, IWUE: irrigation water use efficiency, WD1: mild water deficit in the vegetative stage, WD2: moderate water deficit in the vegetative stage, WD3: severe water deficit in the vegetative stage, WD4: mild water deficit in the vegetative stage and fleshy root growth stage, WD5: mild water deficit in the vegetative stage and moderate water deficit in the fleshy root growth stage, WD6: moderate water deficit in the vegetative stage and mild water deficit in the fleshy root growth stage, WD7: moderate water deficit in the vegetative and fleshy root growth stage, WD8: severe water deficit in the vegetative and mild water deficit in the fleshy root growth stage, WD9: severe water deficit in the vegetative and moderate water deficit in the fleshy root growth stage, CK: normal water supply in all growth stages.

In 2017, the yield of *I. indigotica* was the highest under mild water deficit WD1 (up to 8390.80 kg·ha⁻²), and the yield of *I. indigotica* under other water-deficit treatments was reduced. There were no significant differences in the yield of *I. indigotica* under WD4 and CK, reaching 8235.32 kg·ha⁻². WD3, WD7, WD8, and WD9 were treated with moderate and severe water deficits and showed significant differences compared with CK, decreasing by 18.2%, 18.1%, 31.7%, and 33.4%, respectively. The effects of the water deficit on the yield of *I. indigotica* were similar in 2016 and 2017. WD1 and WD4 had no significant differences compared with CK. However, moderate and severe water deficits significantly reduced the yield compared with CK.
The WUE values of WD1 and WD4 under a mild water deficit were significantly different from CK ($p > 0.05$). The WUE values of WD1 and WD4 were the most significantly different in 2016 ($p < 0.05$), at 8.0% and 8.5% higher than CK, respectively. The WUE values of other treatments were reduced. WD2, WD3, WD7, WD8, and WD9 were significantly lower than CK in terms of WUE, exhibiting decreases of 8.0%, 7.7%, 7.5%, 24.4%, and 25.4%, respectively. In 2017, the WUE of the WD1 treatment was the highest (23.62 kg ha$^{-2}$ mm$^{-1}$) at 8.4% higher than that under CK, followed by WD4, which was 6.7% higher than CK. The effect of a mild water deficit on the WUE of *I. indigotica* was significant. Moderate or severe water deficits resulted in varying degrees of lower crop yield compared with CK.

The IWUE of the WD5, WD8, and WD9 treatments decreased significantly, by rates of 4.2%, 3.9%, and 3.7%, respectively. While mild water-regulation deficits during the vegetative stage significantly improved IWUE, moderate and severe water-regulation deficits during the vegetative stage significantly reduced IWUE.

The WUE of *I. indigotica* under regulated deficit irrigation had a quadratic parabolic relationship with water consumption (Figure 5). The regression models, which are quite similar, are as follows: $y = -0.0031x^2 + 2.2451x - 379.75$, ($R^2 = 0.6825$, Year 2016); $y = -0.0028x^2 + 2.1015x - 366.26$, ($R^2 = 0.6309$, year: 2017). In this regression curve, the WUE of *I. indigotica* showed an overall rising trend, with an increase in water consumption, but when the water consumption reached a critical value (360 mm in 2016 and 369 mm in 2017), the WUE gradually declined. Therefore, to obtain the highest yield and WUE at the same time in this region, the water consumption of *I. indigotica* should be between 355 mm and 370 mm. This means that a high amount of irrigation water and water consumption does not indicate a high WUE.
3.6. Quality

Water deficit influenced the indigo, indirubin, and (R, S)-goitrin contents of *I. indigotica* after the final ripening and harvesting (Table 4). Combining the two-year (2016 and 2017) experimental data indicates that water deficit treatment can increase the content of measured chemical compounds and improve the quality of *I. indigotica*. However, a severe water deficit is not conducive to the accumulation of active ingredients. Due to the impact on (R, S)-goitrin under water-deficit treatments, the contents of (R, S)-goitrin of the WD3, WD8, and WD9 decreased significantly by 8.6% to 13.2% compared with CK ($p < 0.05$). In addition, the content of (R,S)-goitrin was increased in the fleshy root growth stage and the vegetative stage under water deficit. The (R,S)-goitrin content of WD4 and WD5 increased significantly from 5.4% to 7.9%. Because of the impact of water-deficit treatment on the indirubin content, mild and moderate continuous deficits were beneficial to the increase of indirubin content. The differences between WD4, WD5, WD6, and WD7 compared with CK were significant, with an increase of 3.6% to 9.9%. The content of chemical compounds was increased and the
quality improved under water-deficit treatments, and the quality of *I. indigotica* improved as the water deficit intensified. However, a severe water deficit was not conducive to the accumulation of chemical compounds.

Table 4. Impacts of different water-deficit treatments on *Isatis indigotica* quality after the final ripening and harvesting.

| Treatment | Indigo (mg·kg⁻¹) | Indirubin (mg·kg⁻¹) | (R, S)-Goitrin (mg·g⁻¹) | Indigo (mg·kg⁻¹) | Indirubin (mg·kg⁻¹) | (R, S)-Goitrin (mg·g⁻¹) |
|-----------|------------------|---------------------|-------------------------|------------------|---------------------|-------------------------|
| CK        | 6.117 c          | 9.663 c             | 0.237 bc                | 6.121 d          | 9.687 c             | 0.239 c                 |
| WD1       | 6.153 c          | 9.653 c             | 0.230 c                 | 6.139 d          | 9.616 cd            | 0.234 cd                |
| WD2       | 6.093 d          | 9.510 d             | 0.231 bc                | 6.109 d          | 9.594 d             | 0.232 d                 |
| WD3       | 5.737 e          | 8.487 e             | 0.216 d                 | 5.722 e          | 8.474 e             | 0.212 e                 |
| WD4       | 6.463 b          | 9.690 c             | 0.251 a                 | 6.458 b          | 9.788 b             | 0.252 b                 |
| WD5       | 6.670 a          | 10.173 a            | 0.253 a                 | 6.733 a          | 10.195 a            | 0.258 a                 |
| WD6       | 6.443 b          | 9.813 b             | 0.240 b                 | 6.415 bc         | 9.854 b             | 0.249 b                 |
| WD7       | 6.410 b          | 9.807 b             | 0.239 bc                | 6.344 c          | 9.666 cd            | 0.238 cd                |
| WD8       | 5.733 e          | 8.440 e             | 0.208 de                | 5.741 e          | 8.463 e             | 0.210 e                 |
| WD9       | 5.713 e          | 8.463 e             | 0.205 e                 | 5.715 e          | 8.412 e             | 0.208 e                 |

Note: Different lowercase letters within each column indicate significant differences at *p* < 0.05.

4. Discussion

4.1. Leaf Gas Exchange

Water stress can cause a decrease in chlorophyll content, stomatal closure, and starch hydrolysis in leaves, which can slow down the output of photosynthates and affect the photosynthetic physiological process. Severe water stress hinders photosynthesis and affects the dry matter accumulation of crops [20,21]. In maize, a timely and moderate water deficit significantly inhibits the transpiration rate [22]. In addition, a moderate water deficit did not significantly reduce the photosynthetic rate of potato, and photosynthates had a supercompensation effect that was beneficial to the distribution of tubers [23].

The stomatal conductance decreased with water deficit at different growth stages, and the stomatal conductance decreased with the increase of water deficit. For example [24], in grapevine, moderate water stress is normally associated with values of stomatal conductance. After rehydration, the net photosynthetic rate and stomatal conductance of mild water deficit were compensated by rehydration. Compared with CK, all other treatments showed a decrease, ranging from 2.1% to 19.1%, and the decrease was more significant with the intensification of water deficit. Moderate and severe water deficits in the vegetative and fleshy root growth stages of *I. indigotica* inevitably led to the decrease in stomatal opening.

The net photosynthetic rate decreased more significantly as the degree of water deficit intensified. The results showed that the net photosynthetic rates of WD1 and WD4 under mild water deficit increased by 5.5% and 1.8%, respectively, compared with CK in the vegetative stage, and that of WD1 in the fleshy root growth stage increased by 8.5%
compared with CK ($p < 0.05$). Suitable water stress promoted nutrient absorption and enhanced photosynthesis in *I. indigotica*, as with mild water-deficit treatment WD1 and WD4. With the increase in water deficit, the net photosynthetic rates decreased significantly; WD1 and WD4 at the fleshy root growth stage decreased significantly after re-watering. Compared with CK, moderate and severe water deficits significantly reduced the net photosynthetic rate of *I. indigotica* by 7.8% to 19.1%. This was similar to previous research results [25], in which excessive water deficits reduced crop photosynthesis. Previous studies have come to similar conclusions, while further studies also showed that the photosynthetic rate of winter wheat under a severe water deficit decreased significantly, which seriously inhibited its photosynthesis [26]. Although the net photosynthetic rate of *I. indigotica* was affected by water deficit during the vegetative and fleshy root growth stages, the net photosynthetic rate of *I. indigotica* increased after re-watering, demonstrating that re-watering had a strong compensation effect. A mild water deficit helps crops absorb water effectively and thus grow better.

### 4.2. Dry Matter Accumulation

In a previous study, the accumulation of photoassimilative substances was higher under suitable water stress at the tillering stage [27]. In addition, a previous study found that, when the precipitation minus the potential evapotranspiration during the reproductive period (pp-PET RIM) was lower than 70 mm, oil increased linearly with increasing Tm(RSR7) and with rising water deficits [28]. Another study [29] found that the accumulation of dry matter in olive clones decreased with the increase in water deficit. Similar results were obtained in this study. The dry matter accumulation of *I. indigotica* under the WD3, WD8, and WD9 treatments with severe water deficits was significantly reduced by 12.8% to 24.4% compared with CK ($p < 0.05$), while WD1, WD2, and WD4 with mild water deficits were not significantly different from CK during the fleshy root growth stage in terms of dry matter accumulation ($p > 0.05$), and the dry matter accumulation of other treatments was lower than that of CK. The dry matter accumulation of WD1 and WD4 under mild water deficit increased by 8.1% and 6.1%, respectively, compared with CK at fleshy root maturity. The dry matter accumulation of WD5, WD6, and WD7 under moderate water deficit decreased compared with CK, while the dry matter accumulation of treatments under a severe water deficit was significantly lower than CK, with a decrease of 12.5% to 31.1%.

### 4.3. Yield and WUE

In addition to conserving water and increasing yield in fruit trees, regulated deficit irrigation can conserve water and increase yield for maize, rice, wheat, and other field crops [30,31] The results of a previous three-year study showed that all vegetative and yield parameters were significantly affected by water shortages in the soil profile due to regulated deficit irrigation during the sensitive tasseling and cob formation stages [32]. Some scholars have found that a regulated deficit of drip irrigation under mulch can improve the WUE of potatoes, and a mild regulation deficit during tuber formation does not reduce potato yield [33]. Similar results were obtained in this study. Based on the two-year experimental data, under the condition of water shortage in arid and semi-arid areas of northwest China, moderate and timely regulated deficit irrigation of *I. indigotica* can lead to yield increases. If the degree of water deficit in the soil and the corresponding growth period of *I. indigotica* are well controlled, mild water deficit treatment can produce an output close to that under full irrigation. This finding was consistent with research [34] on drip irrigation under mulch in arid areas of northwest China, in which it was found that the appropriate level of water deficit in onions at the seedling and maturity stages was conducive to improving WUE and yield.

With limited irrigation water, a mild water-deficit treatment was found to conserve 15% of the applied irrigation water with no reduction in the crop yield in beans [35]. Similar conclusions were drawn in this study. The WUE of *I. indigotica* was significantly
affected by a regulated water deficit during the vegetative and fleshy root growth stages. The WUE under the WD1 and WD4 treatments with mild water deficits increased significantly ($p < 0.05$) by 6.7% to 8.4% compared with CK, respectively, while the WUE of other treatments decreased. This was mainly because moderate and severe water deficits caused the cell wall to remain firm, inhibiting recovery after re-watering and leading to the reduction of biomass production. High soil moisture leads to the poor aeration of the soil, resulting in the shortened length of main roots.

4.4. Quality of *I. indigotica*

Regulating soil moisture at a certain stage of crop growth promoted the increase of photosynthates and improved product quality. Some scholars have found that under drought conditions, the biomass of *Gynostemma pentaphyllum* decreases while the content of saponins in the leaves increases [36]. In addition, the results of previous research indicate that a water deficit affects flavonoid accumulation in *Scutellaria baicalensis* Georgi, potentially by regulating hormone metabolism [37].

Tan et al. reported that the maximum water demand of *I. indigotica* roots was in July, and the indirubin content was the highest under moderate water stress [11]. In this experiment, the effect of water deficit on the quality of *I. indigotica* was studied. The results showed that both mild and moderate water deficits could increase the contents of indigo and indirubin in *I. indigotica*. The higher the deficit degree, the greater the accumulation of measured chemical compounds. In particular, WD5 significantly increased the contents of indigo, indirubin, and (R, S)-goitrin compared with CK. Different water-deficit treatments had different effects on the content of (R, S)-goitrin, and mild and moderate water deficits could increase the content of (R, S)-goitrin and improve the root quality.

Water-deficit treatment can increase the content of measured tissue chemical composition and improve the quality of *I. indigotica*. The content of measured tissue chemical composition in all treatments reached the pharmacopoeia standard [38]. The quality of *I. indigotica* improved with the intensification of moderate and mild water deficits during the continuous growth period, but a severe water deficit was not conducive to the accumulation of the measured chemical compound (R, S)-goitrin. Severe water deficits in the vegetative stage and severe water deficits in the fleshy root growth period seriously affected the accumulation of (R, S)-goitrin, indigo, and indirubin, while moderate and mild water deficits in these two growth stages increased the accumulation of these chemical compounds to some extent. Therefore, a continuous mild water deficit during the vegetative and fleshy root growth stages is beneficial to the accumulation of (R, S)-goitrin content in *I. indigotica*, but this effect may also be related to genetic and environmental factors.

5. Conclusions

In this study, it was found that a mild water deficit had no significant effect on the growth of *I. indigotica* during the vegetative and fleshy root growth stages; however, moderate and severe water deficits could inhibit the accumulation of dry matter. The net photosynthetic rate of leaves was not significantly affected by mild water deficit but decreased significantly with the increase in water deficit. In addition, a mild water deficit during the vegetative and fleshy root growth stages did not reduce the yield, while moderate and severe water deficits during the vegetative and fleshy root growth stages significantly decreased the yield. As such, mild water-deficit treatment could not only lead to higher yield and WUE but may also increase the contents of indigo, indirubin, and (R, S)-goitrin, which was conducive to improving the quality of *I. indigotica*.

Therefore, reasonable regulated drip irrigation under mulch significantly improved the yield and WUE of *I. indigotica*, enhanced its drought resistance characteristics, and achieved the purpose of high-efficiency, high-yield, and water-efficient agricultural
production. These findings may theoretically guide research on planting *I. indigotica* in this region.

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