Effects of Regulated Deficit Irrigation On Yield and Quality of *Isatis Indigotica* in a Cold and Arid Environment

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Abstract

Field experiments were conducted to study the effects of regulated deficit irrigation on net photosynthetic rate, yield, water use and quality of *Isatis indigotica* under mulched drip irrigation in Hexi oasis region, which can theoretically guide researches on efficient water saving of *Isatis indigotica* planting. In 2016 and 2017, the experiments of regulated deficit irrigation of *Isatis indigotica* were carried out at Yimin Irrigation Experimental Station, Minle County, of Gansu Province, China. Except for sufficient irrigation at seedling and maturity of fleshy roots, *Isatis indigotica* in vegetative stage and fleshy root growth stage were subjected to mild, moderate and severe water deficit respectively, and their photosynthetic physiological indexes, yield and water use were measured. The results showed that the moderate and severe water deficits in vegetative and fleshy root growth stage significantly decreased the net photosynthetic rate and the amounts of dry matter accumulation of *Isatis indigotica* leaves ($P<0.05$), and with the increase of water deficit degree, the decrease of the net photosynthetic rate was greater; while the difference between mild water deficit and control group was not significant ($P>0.05$). The yield and water use efficiency of *Isatis indigotica* subjected to mild water deficit in vegetative and fleshy root growth stage marked the highest, reaching 8,239.56 kg/hm² and 8,390.80 kg/hm², 24.11 kg/hm² and 23.62 kg/hm²·mm, respectively; other water deficit treatments were reduced, which was significantly different from the control group ($P<0.05$). Mild and moderate water deficit could increase the content of effective components ((R, S) - Goitrin, indirubin and indigo), and improve the quality of *Isatis indigotica*, but severe water deficit was not conducive to the accumulation of effective components. Therefore, the optimal water regulation treatment of *Isatis indigotica* is the mild water regulation deficit in vegetative and fleshy root growth stage, which can reduce the water consumption of *Isatis indigotica* and effectively improve the quality and its water use efficiency without reducing its yield.

1 Introduction

Isatis indigotica is one of the commonly used Chinese herbal medicines, which is commonly used for the treatment of cold. Almost all the synergy of Chinese herbal medicines for the treatment of cold includes isatis root. In recent years, it has been found that isatis root and isatis leaf are broad-spectrum plant antibiotics, which are clinically used to treat various inflammation of respiratory, and circulatory systems. In addition, they do have antiviral effect and inhibitory effect on influenza A virus^[1]. The *Isatis indigotica* has been widely introduced in China due to its strong adaptability and low requirements for climate, soil and environmental conditions with Hebei, Shaanxi, Gansu and Jiangsu becoming its main producing areas. However, in the Hexi Corridor, due to the shortage of water resources and unreasonable irrigation methods, the crop yield and water use efficiency are low, which largely restricts the sustainable development of *Isatis indigotica* industry. Therefore, using reasonable irrigation methods and planting methods to improve the yield, water use efficiency and quality of *Isatis indigotica* is the key to solve current problems in *Isatis indigotica* planting. At present, relevant studies abroad show that the technology of regulated deficit irrigation can be applied to tomatoes, lemon trees, citruses, onions and other crops^[2-5] and there are many reports on the application of regulated deficit irrigation in practical production^[6-10], etc., who studied the effect of regulated deficit irrigation on yield and quality of tomato, alfalfa, watermelon, cucumber, wine grape and other crops. The results show that regulated drip irrigation had better water-saving and yield-increasing performances, and can effectively improve crop quality. The studied the effects of different water conditions on the growth and development of *Isatis indigotica* and its main effective components, and found that the yield and quality of *Isatis indigotica* were both excellent when the maximum water-holding capacity was 45%-70%^[11].

The amount of irrigation water and the way of irrigation have certain influence on crop yield and quality. Limited irrigation and irrigation methods effectively improved the 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 used by 5% compared with constant deficit irrigation and improves productivity by 4% for orange trees^[16]. The results revealed that irrigation of sugar beet with drip irrigation method at 75% level (DI25) had significant benefits in terms of saved irrigation water and large WUE, indicating a definitive advantage of deficit irrigation under limited water supply conditions^[17]. The study has revealed that the best sugar rate and root yield of sugar beet is obtained from I1C1 treatment with drip irrigation^[18].

At present, the research on *Isatis indigotica* at home and abroad mainly focuses on its chemical composition, pharmacological activity, plant characteristics, preparation and quality evaluation, etc^[19]. Moreover, the study on regulated water deficit of *Isatis indigotica* at home and abroad only conducted water regulation experiments for the whole growth stage of *Isatis indigotica*, but neglected the growth status, yield and products at different growth stages. In addition, there are few studies on the effect of water deficit on the growth and yield of *Isatis indigotica* under drip irrigation under mulch. Based on the above analysis, it is assumed that water deficit under drip irrigation affects the yield and quality of *Isatis indigotica*. Through field experiment of *Isatis indigotica* in Minle County, Zhangye City, Hexi Corridor, the effects of regulated water deficit on growth dynamics, yield and quality of *Isatis indigotica* in different growth stages of drip irrigation under mulch were discussed. The method of drip irrigation under mulch was further analyzed, which provided theoretical basis for high yield cultivation of *Isatis indigotica* in arid areas of Northwest China.

2 Materials And Methods

2.1 General Description

The experiments were carried out at Yimin Irrigation Pilot Station (100°43'E, 38°39'N) in the middle reaches of Flood River Irrigation District, Minle County, Gansu Province from May to October in 2016 and 2017. The experimental zone has the continental desert steppe climate, with dry climate, abundant heat, abundant light energy and little rain; the altitude is about 1,970 m, the annual average temperature is 6.0°C, $\geq 0^\circ\text{C}$ cumulative temperature is 3500°C, $\geq 10^\circ\text{C}$ effective cumulative temperature is 2,985°C, the extreme maximum temperature is 37.8°C, the extreme minimum temperature is - 33.3°C, the average sunshine hours is 3,000 hours for years, and the frost-free period is 125 days. According to the data of precipitation for years, the average annual precipitation in this area is 215 mm with little precipitation and large variation rate. The contradiction between supply and demand is prominent, and the drought is frequent. The soil is light loam with pH value of 7.22, field water holding capacity of tillage layer soil is 24%, soil bulk density is $1.4\text{ g}\cdot\text{cm}^{-3}$, groundwater level is low, and the area does not show salinization and alkalization.

2.2 Test Materials and Cultivation Methods

The large, full and even *Isatis indigotica* seeds, were independently planted in the Department of Chinese herbal medicine in the Gansu Agricultural University, of which the seed purity was 96%, the weight per 1,000 seeds was 9.873 g, the germination rate was 87.6%, and the germination potential was 46.4%. Sowed on May 3 and harvested on October 13, the seeds have the sowing amount of $30.0\text{ kg}\cdot\text{hm}^{-2}$ and the planting density was $700,350\text{ plants}\cdot\text{hm}^{-2}$. Before sowing, the experimental zone was ploughed for 30 cm to remove weeds manually.

At the same time, 210 kg·hm⁻² urea (46% of N) was applied, 340 kg·hm⁻² calcium superphosphate (12% of P₂O₅, 10% of S, 16 % of Ca) was applied, and 270 kg·hm⁻² source potassium (25% of K₂O) was applied. All fertilizers were applied as base fertilizer at the time of sowing. Before *Isatis indigotica* were planted, three drip irrigation belts were laid manually in each plot with a distance of 1 m, a distance of 30 cm among 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, pressure gauge and water meter were located at the drip irrigation hub, and the working pressure of the system was 0.1 MPa. After drip irrigation system was installed, the soil was covered with colorless plastic film with a width of 120 cm. Each experimental plot is separated by a film with a width of 60 cm to prevent water from seepage underground. The experimental research and field studies on plants, including the collection of plant material were in accordance with the Irrigation experiment standard (SL 13–2015) issued by the Water Industry Standards of the People's Republic of China. The permission for collecting of 'Isatis indigotica' were approved by Yimin Irrigation Experimental Station and Flood River Administration Office,Minle CountyChina.

2.3 Experiment Design

The growth stage of *Isatis indigotica* is divided into four growth stages according to its growth characteristics: seedling stage (May 3-June 7), vegetative stage (June 8-July 18), fleshy root growth stage (July 19-August 28) and fleshy root maturity (August 29-October 13). Soil moisture is divided into four levels: adequate irrigation (F, soil water content is 75%-85% of field water holding capacity), mild water deficit (L, soil water content is 65%-75% of field water holding capacity), moderate water deficit (M, soil water content is 55%-65% of field water holding capacity), and severe water deficit (H, soil water content is 45%-55% of field water holding capacity). There are 10 water control treatments, of which CK is the control treatment. Each treatment is repeated three times, totaling 30 districts. The area of each district is 36 m² (9 m × 4 m) and random block design is adopted. The effective experimental planting area is 1080 m². The method of irrigation is drip irrigation under mulch. The depth of soil layer for soil moisture control is 100 cm during the whole growth stage of irrigation. The upper and lower limits of water control coincide with the regional reality. The specific experimental design is shown in Table 1.

Table 1
Soil Moisture Contents under Different Treatments (Percentage of field water-holding capacity)

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

2.4 Indicators and Methods for Measurement

2.4.1 Dry matter and yield

From the emergence of *Isatis indigotica* to the end of each growth period, four measurements were made. Five *Isatis indigotica* 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 organs were cut off from the plant, washed and dried indoors, marked with paper bags and packed separately. The temperature of *Isatis indigotica* was fixed at 105°C for 30 minutes, then the temperature was adjusted to 80°C, and dried to constant weight. The above-ground and underground dry matter were measured by electronic weighing with a precision of 0.001 g. The results were taken as the average values of each treatment. After the fleshy roots of *Isatis indigotica* were mature, 20 *Isatis indigotica* seedlings were harvested and brought back to the room for washing and drying, and the total yield per hectare was gained after final conversion.

2.4.2 Photosynthetic characteristics

The net photosynthetic rate (P_n) of leaves was measured at 9:00–11:00 a.m. on the 7th day after regulated water deficit treatment with LI-6400 portable photosynthesizer under the photon intensity of $1,000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The middle part of the third functional leaf from inside to outside was selected as the measuring part and labeled well. Five plants were measured in each plot. The average value of each treatment was calculated.

2.4.3 Water use efficiency (WUE) and irrigation water use efficiency(IWUE)

$$WUE = Y/ET_a$$

$$IWUE = Y/I$$

where WUE is water use efficiency ($\text{kg}/\text{hm}^2\cdot\text{mm}$), $IWUE$ is irrigation water use efficiency ($\text{kg}/\text{hm}^2\cdot\text{mm}$), Y is yield per unit area of *Isatis indigotica* ($\text{kg}/\text{hm}^2\cdot\text{mm}$), ET_a is water consumption (mm) and I is irrigation water amount (mm) during the whole growth period of *Isatis indigotica*.

2.4.4 Quality measurement

Sample preparation: Samples of *Isatis indigotica* were collected, washed, wetted and sliced after impurities were removed, then they were dried to constant weight by blast at 60°C in oven, crushed and sifted through 40 meshes. The same batch of isatis root powder for 1.0 g (electronic balance) was accurately weighed and placed in conical bottle. 50 mL of deionized water was added, weighed and recorded. After 50 minutes 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. They were well shaken and then filtered with 0.45 μm membrane. The sample solution then was be obtained. Six samples were prepared simultaneously for each sample.

High performance liquid chromatography (LC-10AT_{VP}, Shimadzu Corporation, Japan) chromatographic conditions: SPD-10Avp (UV-VIS) detector, chromatographic column of Agilent Zorbax SB-C18 (100 mm x 4.6 mm, 3.5 μm), methanol-0.1% formic acid solution taken as mobile phase; flow rate is 1.0 $\text{mL}\cdot\text{min}^{-1}$ with automatic sampler injection, and injection volume is 20 μL ; detection wave is 280 nm long and the temperature of the column is 25°C. Indigo, indirubin, (R, S) - goitrin standard products are provided by National Institutes for Food and Drug Control.

The preparation of reference solution: The electronic balance weighed indigo standard for 1.0 mg, indirubin standard for 1.0 mg, (R, S) - goitrin standard for 1.0 mg, and they were placed in a 100 mL bottle, dissolved in appropriate amount with ethanol, diluted with ethanol to the scale, shaken evenly, and then indirubin, indigo, and (R, S) - goitrin reference solution was made. 0.5 mL of the reserve solution in a 10 mL measuring cylinder was measured precisely, which was diluted with ethanol to the scale and well shaken. Then 0.5 $\mu\text{g}\cdot\text{mL}^{-1}$ reference solution was made.

Sample content measurement: 20 mL of reference solution and sample test solution was precisely measured and absorbed with samples injected constantly for 6 times. Chromatogram was recorded and peak area calculated. The content of indirubin, indigo, (R, S) – goitrin was calculated according to external standard method.

2.5 Data Analysis

EXCEL 2010 was used to calculate the measured data. Duncan multiple comparison method in SPSS 19.0 software was used to compare the significance of the difference between the data processed. GraphPad Prism 5.01 was used to draw the graph. The data in each table were average values of three replicates.

3 Results

3.1 Dynamics of temperature and precipitation in the whole plant growth periods

Although the daily average temperature of *Isatis indigotica* fluctuates in a certain range during the whole growth period, the fluctuation was similar to the local temperature variation in the past five years (Fig. 1). In 2016, the lowest daily average temperature was 12.8°C, which appeared at the beginning of seedling stage, and the highest daily average temperature was 27.6°C, which appeared at the fleshy root growth stage. The average daily temperature of *Isatis indigotica* during the whole growth period in 2017 was 3.75°C at the beginning of seedling stage and 2.425°C at the later maturity of fleshy roots, respectively. The maximum daily average temperature was 29.8°C at fleshy root growth stage. In 2017, the total precipitation of *Isatis indigotica* was 185.8 mm. The precipitation mainly concentrated in the fleshy root growth stage and the maturity, of which the amount reached 72.8 mm and 60.5 mm respectively, accounting for 39.18% and 32.56% of the total precipitation of the whole growth period. The precipitation of *Isatis indigotica* in 2017 was 196.5mm 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.

3.2 Net photosynthetic rate

Net photosynthetic rate of *Isatis indigotica* changed marginally among treatments at seedling stage and fleshy root maturity stage, but water deficit showed more significant effect on net photosynthetic rate at vegetative stage and fleshy root growth stage (Fig. 2). In 2016, net photosynthetic rate of all treatments was smaller than that of the control group at vegetative stage ($p > 0.05$). Among them, WD3 and WD9 net photosynthetic rates decreased by 17.1% and 19.1% respectively compared with CK ($p < 0.05$). The compensatory effect occurred under WD1 and WD4 after the samples were re-watered at fleshy root growth stage. The net photosynthetic rate reached $19.62 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and $19.84 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ percent 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 of moderate water deficit decreased respectively at fleshy root growth stage, but it recovered after the samples were re-watered, which was just lower than that of CK, reaching 3.82% and 4.75% respectively. In 2017, the net photosynthetic rates of WD1 and WD4 increased by 5.51% and 1.82% respectively compared with CK at vegetative stage; other treatments were reduced from 2.2–15.6%. The net photosynthetic rate of WD1 increased by 8.48% compared with CK at fleshy root growth stage; other treatments were reduced from 0.5–19.4%. The net photosynthetic rate decreased more significantly as the degree of water deficit intensified.

That indicates that though affected by water deficit at vegetative stage and fleshy root growth stage, the net photosynthetic rate recovers after *Isatis indigotica* is re-watered at fleshy root growth stage and fleshy root maturity, showing a greater water compensation effect.

3.3 Dry Matter Accumulation

Dry matter accumulation in different growth stages of *Isatis indigotica* under drip irrigation under mulch is shown in Fig. 3. The dry matter accumulation process of *Isatis indigotica* showed a “S”-shaped curve during the whole growth period. From seedling stage to vegetative stage, the plant growth process and the dry matter accumulation were slow; from vegetative stage to fleshy root growth stage, the plant grew vigorously and the dry matter accumulation was rapid; from fleshy root growth stage to fleshy root maturity, the plant growth was slow while dry matter accumulation increased steadily. In 2016, the highest dry matter accumulation per plant under WD1 was 7.38 (g/plant) in vegetative period, and there was no significant difference between WD1 and CK ($p > 0.05$) while other treatments were lower than CK. The dry matter under WD3, WD7, WD8 and WD9 treatments was significantly lower than CK, and the reduction ranged from 12.8–17.6%. In 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. Those of WD1 and WD4 treatments significantly increased 8.1% and 6.1% compared with that of CK at fleshy root maturity, and those of moderate and severe water deficit treatments were significantly lower than that of CK, with a decrease ranging from 12.5–31.1%. In 2017, there was no significant difference between mild and moderate water deficit treatments, and CK in vegetative period. WD3, WD8 and WD9 with severe water deficit significantly decreased by 18.3%, 23.1% and 24.4% compared with CK; WD1, WD2 and WD4 had no significant difference with CK in fleshy root growth stage in terms of dry matter accumulation, while the other treatments significantly decreased compared with CK; WD1 increased by 4.0% compared with CK in fleshy root maturity, but there was no significant difference.

The dry matter accumulation processes of *Isatis indigotica* in 2016 and 2017 were similar. There was no significant difference in dry matter accumulation of *Isatis indigotica* during the growth stage due to no water deficit treatment at seedling stage ($p > 0.05$). However, the dry matter accumulations of *Isatis indigotica* in 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 had been formed in the fleshy root maturity stage. The dry matter accumulation of *Isatis indigotica* showed a “S”-shaped curve with time passing by during the whole growth period, but severe water deficit would significantly reduce the dry matter accumulation of *Isatis indigotica*, while timely and appropriate mild water deficit would not significantly affect the dry matter accumulation, and was conducive to improving the water use efficiency of crops.

3.4 Yield

In 2016, yield of *Isatis indigotica* of CK was the highest under the condition of full irrigation (up to 8,315.58kg /hm²) in the whole growth period, and yield of *Isatis indigotica* under other water deficit treatments was reduced (Table 2). WD1 and WD4 showed no significant difference compared with CK ($p > 0.05$), reaching 8,239.56kg/hm² and 8,215.52 kg/hm² respectively. WD3, WD7, WD8 and WD9 treated with moderate and severe water deficit showed significant difference compared with CK ($p < 0.05$), decreased respectively by 17.09 %, 16.23 %, 36.13 % and 37.12 %.

In 2017, yield of *Isatis indigotica* was the highest under the condition of full irrigation WD1 (up to 8,390.80kg /hm²), and yield of *Isatis indigotica* under other water deficit treatments was reduced. There was no significant difference between yield of *Isatis indigotica* under WD4 and CK, reaching 8,235.32kg /hm². WD3, WD7, WD8 and WD9 treated with moderate and severe water deficit showed significant difference compared with CK, decreased respectively by 18.29%, 18.05%, 31.67% and 33.43%. The effects of water deficit on yield of *Isatis indigotica* were similar in 2016 and 2017. WD1 and WD4 had no significant difference compared with CK. However, moderate and severe water deficit significantly reduced the yield compared with CK.

3.5 Water use efficiency and irrigation water use efficiency

Water use efficiency (WUE) of WD1 and WD4 under the condition of mild water deficit was significantly different ($p > 0.05$). In 2016, WUE of WD1 and WD4 showed the most significance ($p < 0.05$), which was 8.00% and 8.45% higher than CK. WUE of other treatments was reduced. WD2, WD3, WD7, WD8 and WD9 were significantly lower than CK in terms of WUE, which decreased by 8.01%, 7.69%, 7.45%, 24.38% and 25.41%, respectively. In 2017, WUE of WD1 treatment was the highest (23.62kg/hm²•mm), 8.35% higher than that under CK, followed by WD4, 6.74% higher than CK. The effect of mild water deficit on WUE of *Isatis* was significant.

The irrigation water use efficiency of WD5, WD8 and WD9 treatment decreased significantly, with a rate of 4.19%, 3.91% and 3.73%, respectively. Mild water regulation deficit during Vegetative stage significantly improved the irrigation water use efficiency, while moderate and severe water regulation deficit during Vegetative stage significantly reduced the irrigation water use efficiency.

Table 2
Effect of Different Water Deficit on Yield and WUE of *Isatis indigotica*

Year	Treatment	Precipitation/mm	Total Water Consumption/mm	Yield/kg·hm ⁻²	IWUE kg/hm ² ·mm	WUE/kg·hm ⁻² ·mm ⁻¹
2016	CK	185.8	374.04	8315.58a	50.94b	22.23b
	WD1	185.8	343.28	8239.56a	54.04a	24.01a
	WD2	185.8	353.05	7219.67b	49.03c	20.45d
	WD3	185.8	335.92	6894.60d	51.03b	20.52d
	WD4	185.8	340.85	8215.52a	54.67a	24.11a
	WD5	185.8	346.06	7164.91bc	49.32c	20.70cd
	WD6	185.8	338.38	7083.69c	49.68c	20.93c
	WD7	185.8	338.56	6965.85d	50.57b	20.57d
	WD8	185.8	316.03	5311.57e	46.10d	16.81e
	WD9	185.8	315.27	5228.54e	46.48d	16.58e
2017	CK	196.5	381.75	8322.25a	50.36bc	21.80b
	WD1	196.5	355.25	8390.80a	54.57a	23.62a
	WD2	196.5	366.06	7462.24b	49.89c	20.39c
	WD3	196.5	343.62	6800.36e	51.47b	19.79d
	WD4	196.5	353.93	8235.32a	54.03a	23.27a
	WD5	196.5	357.65	7051.11c	48.25d	19.72d
	WD6	196.5	348.66	6981.71cd	49.11cd	20.02cd
	WD7	196.5	347.35	6819.79de	50.20bc	19.63d
	WD8	196.5	329.02	5686.71f	48.39d	17.28e
	WD9	196.5	327.78	5539.79f	48.48d	16.90e
Note: Within each column different small letters mean significant difference at $p < 0.05$.						

3.5 Quality

Indigo, indirubin and (R, S)-goitrin content of *Isatis indigotica* were affected by water deficit during the vegetative stage and fleshy root growth stage (Table 3). Combining the two-year (2016 and 2017) experimental data, the water deficit treatment can increase the content of active ingredients and improve the quality of *Isatis indigotica*. However, the severe water deficit is not conducive to accumulation of active ingredients. Due to the impact of (R, S)-goitrin under water deficit treatment, the content (R, S)-goitrin of WD3 and WD8, WD9 decreased significantly by 8.59–13.23% compared with CK ($p < 0.05$). In addition, the content (R,S)-goitrin was increased in fleshy root growth stage and vegetative stage under water deficit. WD4 and WD5 significantly increased by 5.43–7.94%. Because of impact of indirubin on water deficit treatment, the mild and moderate continuous deficit is beneficial to the

increase of indirubin content. The differences between WD4, WD5, WD6 and WD7 compared with CK were significant, with the increase of 3.6–9.9%. The content of effective components was increased and the quality improved under water deficit treatments. The content of effective components under each treatment reached the pharmacopoeia standard. The quality of *Isatis indigotica* was improved as water deficit intensifies. However, severe water deficit is not conducive to the accumulation of effective components.

Table 3
Impact of Different Water Deficit Treatments on Quality of *Isatis indigotica*

	2016			2017		
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	6.117cd	9.663c	0.237bc	6.121d	9.687c	0.239c
WD1	6.153c	9.653c	0.230c	6.139d	9.616cd	0.234cd
WD2	6.093d	9.510d	0.231bc	6.109d	9.594d	0.232d
WD3	5.737e	8.487e	0.216d	5.722e	8.474e	0.212e
WD4	6.463b	9.690c	0.251a	6.458b	9.788b	0.252b
WD5	6.670a	10.173a	0.253a	6.733a	10.195a	0.258a
WD6	6.443b	9.813b	0.240b	6.415bc	9.854b	0.249b
WD7	6.410b	9.807b	0.239bc	6.344c	9.666cd	0.238cd
WD8	5.733e	8.440e	0.208de	5.741e	8.463e	0.210e
WD9	5.713e	8.463e	0.205e	5.715e	8.412e	0.208e
Note: Within each column different small letters mean significant difference at $p < 0.05$.						

4 Discussion

4.1. Net Photosynthetic Rate

Water stress can cause the decrease of chlorophyll content, stomatal closure and starch hydrolysis in leaves, which can slow down the output of photosynthate and affect the photosynthetic physiological process. Furthermore, the chloroplast structure is damaged and photosynthesis is hindered, thus affecting the accumulation of photosynthate^[20–21]. However, timely and moderate water deficit significantly inhibits the transpiration rate of maize. In addition, the decrease of photosynthetic rate is not significant, as there are super-compensation effects in photosynthate, which is conducive to the distribution to grains^[22].

The results showed that the net photosynthetic rates of WD1 and WD4 under mild water deficit increased by 5.5% and 1.8% compared with CK in vegetative stage, and that of WD1 in the fleshy root growth stage increased by 8.5% compared with CK ($p < 0.05$). With the increase of water deficit, the net photosynthetic rates decreased

significantly; WD1 and WD4 at fleshy root growth stage decreased significantly after re-watering. Compared with CK, moderate and severe water deficit significantly reduced the Pn of *Isatis indigotica* by 7.8–19.1%, which was similar to the results gained [23]. Previous studies have similar conclusions, other studies also showed that the photosynthetic rate of winter wheat under severe water deficit decreased significantly, which seriously inhibited its photosynthesis [24]. Although the Pn content of *Isatis indigotica* was affected by water deficit during vegetative and fleshy root growth stages, the net photosynthetic rate of *Isatis indigotica* increased after re-watering, showing a strong compensation effect of re-watering.

4.2 Dry Matter Accumulation

Photoassimilates accumulated in the third internode of the upper part of the main stems, as well as in the flag leaf sheath, are mobilized in a higher proportion and can contribute to grain filling in rice plants subjected to water stress in the tillering phase [25]. The study found that when precipitation minus potential evapotranspiration during the reproductive period (pp-PET RIM) was lower than 70 mm, oil increased linearly with increasing Tm(R5R7) and with rising water deficit [26]. Meanwhile, protein increased linearly with Tm(R5R7) but decreased linearly with water deficit. Levy David [27] found that the accumulation of dry matter in potato decreased with the increase of water deficit in seedling stage. Similar results were also obtained in this study. Dry matter accumulation of *Isatis indigotica* under WD3, WD8 and WD9 treatments with severe water deficit was significantly reduced by 12.8–24.4% compared with CK under full irrigation ($p < 0.05$), while WD1, WD2 and WD4 with mild water deficit were not significantly different from CK during fleshy root growth stage in terms of dry matter accumulation ($p > 0.05$), and those of other treatments were lower than that of CK. The dry matter accumulation of WD1 and WD4 under mild water deficit treatment increased by 8.1% and 6.1% compared with CK at fleshy root maturity. WD5, WD6 and WD7 under moderate water deficit treatment decreased compared with CK, while those under severe water deficit treatment were significantly lower than CK, with a decrease of 12.5–31.1%.

4.3 Yield and Water Use Efficiency

Regulated deficit irrigation cannot only be used to save water and increase yield with fruit trees and pear trees, but also save water and increase yield for maize, rice, wheat and other field crops [28–29]. Results of this 3-year study show that all vegetative and yield parameters were significantly affected by water shortage in the soil profile due to omitted irrigation during the sensitive tasselling and cob formation stages [30]. The study found that regulated deficit of drip irrigation under mulch could improve water use efficiency of potatoes, and mild regulation deficit during tuber formation would not reduce potato yield [31]. 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 *Isatis indigotica* can lead to the yield increase. At the same time, if the degree of water deficit in soil and the corresponding growth period of *Isatis indigotica* are well controlled, mild water deficit treatment can produce the output close to that under full irrigation. This is consistent with the research [32]. Under drip irrigation under mulch in arid areas of Northwest China, a certain water deficit of onion at seedling and maturity is conducive to improving water use efficiency and yield.

From Fig. 4, it can be seen that the water use efficiency of *Isatis indigotica* under regulated deficit irrigation has a quadratic parabolic relationship with water consumption. The regression models are: $y = -0.0031x^2 + 2.2451x - 379.75$, ($R^2 = 0.6825$, Year 2016); $y = -0.0028x^2 + 2.1015x - 366.26$, ($R^2 = 0.6309$, Year 2017), which are quite similar. In this regression curve, the water use efficiency of *Isatis indigotica* shows an overall rising trend with the increase of water consumption, but when the water consumption reaches a critical value (360 mm in 2016, 369

mm in 2017), the water use efficiency gradually declines. Therefore, in order to obtain the highest yield and water use efficiency at the same time in this area, the water consumption of *Isatis indigotica* should be between 355 mm and 370 mm. It means that the high amount of the irrigation water and water consumption does not indicate the high water-use efficiency.

Under limited irrigation water, mild water deficit treatment was found to be favorable to save 15% of the applied irrigation water with no reduction in bean crop [33]. Similar conclusions were drawn in this study. Water use efficiency of *Isatis indigotica* was significantly affected by regulated water deficit during vegetative and fleshy root growth stages. Water use efficiency under WD1 and WD4 treatments with mild water deficit increased significantly ($p < 0.05$), increased by 6.74–8.45% compared with CK, while water use efficiency of other treatments decreased. This is mainly because moderate and severe water deficit allows the cell wall to be firm, which cannot be recovered easily after re-watering, leading to the reduction of biomass production; high soil moisture leads to poor aeration of soil, resulting in the shortened length of main roots, increased lateral roots and decreased medicinal value; little soil moisture can intensify the lignification of roots, and it is also not conducive to root growth.

4.4 Quality of *Isatis indigotica*

Water is the medium for improving crop quality. It can improve crop metabolism, promote the increase of photosynthate and improve the quality of products by regulating soil moisture in some growing stages of crops. With the development of large-scale planting of Chinese medicinal herbs, the regulation of soil moisture on the growth, yield and quality of medicinal herbs has been paid more and more attention by researchers. The study found that under drought conditions, the biomass of *Gynostemma pentaphyllum* decreased while the content of saponins in leaves increased [34]. The results indicate that water deficit affected flavonoid accumulation might through regulating hormone metabolism in *S. baicalensis* Georgi [35].

Tan Yong confirmed that the maximum water demand of *Isatis* roots was in July, and the content of indirubin in *Isatis* roots was the highest under moderate water stress [11]. In this experiment, the effect of water deficit on the quality of *Isatis indigotica* was studied. The results showed that both mild and moderate water deficit could increase the contents of indigo and indirubin in *Isatis indigotica*. The higher the deficit degree, the more the accumulation of effective components. 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 in *Isatis indigotica*, and mild and moderate water deficit could increase the content of effective ingredient (R, S) – goitrin in *Isatis indigotica* and improve the quality of *Isatis indigotica*. The content of effective ingredient in all treatments met the pharmacopoeia standard.

Water deficit treatment can increase the content of effective components and improve the quality of *Isatis indigotica*. The content of effective components in all treatments reaches the pharmacopoeia standard [36]. With the intensification of moderate and mild water deficit degree in continuous growth period, the quality of *Isatis indigotica* is improved, but severe water deficit is not conducive to the accumulation of content of effective components (R, S) -goitrin. Severe water deficit in vegetative stage and severe water deficit in fleshy root growth period will seriously affect the accumulation of effective components (R, S) -goitrin, indigo and indirubin, and the moderate and mild water deficit in these two growth stages can accumulate effective components to a certain extent. Therefore, continuous mild water deficit during vegetative and fleshy root growth stages is beneficial to the accumulation of (R, S) -goitrin content in *Isatis indigotica*, but it may also be related to its own genetic factors and ecological environment.

5 Conclusions

The mild water deficit had no significant effect on the growth of *Isatis indigotica* during vegetative and fleshy root growth stages, but moderate and severe water deficit could inhibit the accumulation of dry matter in *Isatis indigotica*. The net photosynthetic rate of *Isatis indigotica* leaves was not significantly affected by mild water deficit, but decreased significantly with the increase of water deficit. Mild water deficit during vegetative and fleshy root growth stages did not reduce the yield of *Isatis indigotica*, while moderate and severe water deficit during other vegetative and fleshy root growth stages significantly decreased the yield of *Isatis indigotica*. That is to say, mild water deficit treatment could not only lead to higher yield and water use efficiency, but also the same contents of indigo, indirubin and goitrin, which is conducive to improving the quality of *Isatis indigotica*.

Therefore, reasonable regulated drip irrigation under water mulch can significantly improve the yield and water use efficiency of *Isatis indigotica*, enhance its drought resistance characteristic, achieve the purpose of high efficiency, high yield and water saving effect, which can theoretically guide researches on planting *Isatis indigotica* in this area. However, due to the limited experimental conditions, further studies need to be conducted on the yield and water use of *Isatis indigotica* under the combined effects of water treatment and fertilizers.

Declaration

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We would like to declare on behalf of all authors of this manuscript that we have permission to collect 'Isatis indigotica'. This permission comes from Yimin Irrigation Experimental Station, Flood River Administration Office. Minle County, China. Changlong Zhang is the administrator of this institution.

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References

1. Yuan, H. U. A. N. G., Jing, L. I., Keyi, X. U., Deqin, W. A. N. G. & Zifeng, Y. A. N. G. Research Progress on Anti-influenza Effective Components of Isatidis Radix. *Chinese Journal of Modern Applied Pharmacy*. **36** (20), 2618–2623 (2019).
2. Sladjana, S. *et al.* Comparative effects of regulated deficit irrigation (rdi) and partial root-zone drying (prd) on growth and cell wall peroxidase activity in tomato fruits. *Sci. Hort.* **117** (1), 0–20 (2008).
3. Pérez, J. G. *et al.* Comparison of deficit and saline irrigation strategies to confront water restriction in lemon trees grown in semi-arid regions. *Agricultural Water Management*. **164**, 46–57 (2016).

4. María, R. C. *et al.* Effects of deficit irrigation applied during fruit growth period of late mandarin trees on harvest quality, cold storage and subsequent shelf-life[J]. *Sci. Hort.* **165**, 344–351 (2014).
5. Igbadun, H. E., Ramalan, A. A. & Oiganji, E. Effects of regulated deficit irrigation and mulch on yield, water use and crop water productivity of onion in samaru, nigeria. *Agric. Water Manage.* **109**, 162–169 (2012).
6. Gan*, H. Z. Y., Huang, G. & Zhao, W. Fengmin Li, 2009. Postharvest residual soil nutrients and yield of spring wheat under water deficit in arid northwest China. *Agricultural Water Management*. 96, 1045–1051.
7. Wang, H. *et al.* Coupling effects of water and fertilizer on yield, water and fertilizer use efficiency of drip-fertigated cotton in northern xinjiang, china. *Field Crops Research.* **219**, 169–179 (2018).
8. Kou, D., Su, D., Wu, D. & Li, Y. Effects of regulated deficit irrigation on water consumption, hay yield and quality of alfalfa under subsurface drip irrigation. *Nongye Gongcheng Xuebao/Transactions of the Chinese Society of Agricultural Engineering.* **30** (2), 116–123 (2014).
9. Zhang, L. *et al.* Rubisco gene expression and photosynthetic characteristics of cucumber seedlings in response to water deficit. *Sci. Hort.* **161** (Complete), 81–87 (2013).
10. He, J., Wang, Z., He, X. & & The influence of different irrigation water on grapes growth and yield under drip irrigation in extremely arid regions. *Journal of Agriculture.* **3**, 65–69 (2013).
11. Yong, T. A. N., Zongsuo, L. I. A. N. G., Juane, D. O. N. G., Haiyuan, H. A. O. & YE Qing. Effect of stress on growth and accumulation of active components of *Isatis indigotica*. *China Journal of Chinese Materia Medica.* **33** (1), 19–22 (2008).
12. Rop, D. K., Kipkorir, E. C. & Taragon, J. K. (2016). Effects of deficit irrigation on yield and quality of onion crop. *Journal of Agricultural Science*, 8(3).
13. Yang, L., Qu, H., Zhang, Y. & Li, F. Effects of partial root-zone irrigation on physiology, fruit yield and quality and water use efficiency of tomato under different calcium levels. *Agric. Water Manage.* **104** (none), 89–94 (2012).
14. Huang, C. H. *et al.* Impact of saline water irrigation on yield and quality of melon (*cucumis melo* cv. huanghemi) in northwest china. *European Journal of Agronomy.* **43** (none), 68–76 (2012).
15. Amer, K. H. Effect of irrigation method and quantity on squash yield and quality. *Agric. Water Manage.* **98** (8), 1197–1206 (2011).
16. Antonio Jesús Zapata-Sierra, & & Manzano-Agugliaro, F. (2017). Controlled deficit irrigation for orange trees in mediterranean countries. *Journal of Cleaner Production.* 162 (2017) 130–140
17. Ramazan Topak • Sinan Su`heri • Bilal Acar. Effect of different drip irrigation regimes on sugar beet (*Beta vulgaris* L.) yield, quality and water use efficiency in Middle Anatolian, Turkey. *IRRIGATION SCIENCE.* **29**, 79–89 (2011).
18. Kiymaz, S. Water use and yield of sugar beet (*beta vulgaris* l.) under drip irrigation at different water regimes. *Agric. Water Manage.* **158** (12), 225–234 (2015).
19. BAI Yu, T. A. N. G. *et al.* Effect of Nitrogen Nutrition on Growth and Accumulation of Active Ingredients in *Isatis indigotica* Fort. *Journal of Nuclear Agricultural Sciences.* **31** (1), 169–178 (2017).
20. Xu, W. Z., Deng, X. P. & Xu, B. C. Effects of water stress and fertilization on leaf gas exchange and photosynthetic light-response curves of *bothriochloa ischaemum* l. *Photosynthetica.* **51** (4), 603–612 (2013).
21. Dwivedi, S. K., Arora, A. & Kumar, S. Paclobutrazol-induced alleviation of water-deficit damage in relation to photosynthetic characteristics and expression of stress markers in contrasting wheat genotypes. *Photosynthetica.* **55** (2), 351–359 (2016).

22. Li, Y. P., Li, Y. Y., Li, D. Y., Wang, S. W. & Zhang, S. Q. Photosynthetic response of tetraploid and hexaploid wheat to water stress. *Photosynthetica*. **55** (3), 1–13 (2016).
23. Tian, F., Jia, T. & Yu, B. Physiological regulation of seed soaking with soybean isoflavones on drought tolerance of glycine max, and glycine soja. *Plant. Growth Regul.* **74** (3), 229–237 (2014).
24. Sharma, M., Gupta, S. K., Majumder, B., Maurya, V. K. & Pandey, V. Salicylic acid mediated growth, physiological and proteomic responses in two wheat varieties under drought stress. *Journal of proteomics*. **163**, 28 (2017).
25. García, A., Dorado, M., Pérez, I. & Montilla, E. (2010). Effect of water deficit on the distribution of photoassimilates in rice plants (*oryza sativa* L.). *Interciencia*, 35(1).
26. Carrera, C., María José Martínez, Dardanelli, J. & Mónica Balzarini. &. (2009). Water deficit effect on the relationship between temperature during the seed fill period and soybean seed oil and protein concentrations. *Crop Science*, 49(3).
27. Levy, D. Water deficit enhancement of proline and amino nitrogen accumulation in potato plants and its association with susceptibility to drought. *Physiol. Plant.* **57** (1), 169–173 (2006).
28. Kang, S., Shi, W. & Zhang, J. An improved water-use efficiency for maize grown under regulated deficit irrigation. *Field Crops Research*. **67** (3), 0–214 (2000).
29. Bouazzama, B., Xanthoulis, D., Bouaziz, A., Ruelle, P. & Mailhol, J. Effect of water stress on growth, water consumption and yield of silage maize under flood irrigation in a semi-arid climate of tadla (morocco). *Biotechnologie Agronomie Société Et Environnement*. **16** (4), 468–477 (2012).
30. Recep Çakir. Effect of water stress at different development stages on vegetative and reproductive growth of corn. *Field Crops Research*. **89** (1), 1–16 (2004).
31. Zhang Hengjia Li Jing. (2013). Photosynthetic Physiological Characteristics and Water Use of Potato with Mulched Drip Irrigation under Water Deficit in Oasis Region [J]. *Nongye Gongcheng Xuebao/Transactions of the Chinese Society of Agricultural Engineering*, 2013, 44(10):143–151.
32. Zheng, J., Huang, G., Huang, Q., Wang, J. & Zheng, K. Water production function and optimal irrigation schedules for onion with drip irrigation and mulch of plastic film in arid region. *Nongye Gongcheng Xuebao/Transactions of the Chinese Society of Agricultural Engineering*. **27** (8), 25–30 (2011).
33. Abd El-Wahed, M. H., Baker, G. A., Ali, M. M., El-Fattah, A. & F. A *Effect of drip deficit irrigation and soil mulching on growth of common bean plant, water use efficiency and soil salinity* (SCIENTIA HORTICULTURAE, 2017).
34. Yun, L. O. N. G., Rui, Y. A. N. G., Zhang-cheng, Z. H. O. N. G. & Feng, T. A. N. Effect of different water and nitrogen on biomass and gypenosides in *Gynostemma pentaphyllum*. *Chinese Traditional and Herbal Drugs*. **39** (12), 1872–1876 (2008).
35. Yuan, Y. *et al.* Water deficit affected flavonoid accumulation by regulating hormone metabolism in *scutellaria baicalensis georgi* roots. *Plos One*. **7** (10), (2012). e42946
36. National pharmacopoeia committee. *Chinese Pharmacopoeia Commission[M]* (China medical science and technology press, Beijing, 2015).

Figures

Figure 1

Average Temperature and Precipitation Changes during Different Growth Stages

Figure 2

Photosynthetic Rate under Various Water Treatments across the 2016 and 2017 Cropping Seasons

Figure 3

Dry Matter Accumulation of Single Plant under Various Regulated Deficit Treatments

A **B** **Figure 4**

Relationship between Yield and Water Use Efficiency and Water Consumption