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Effects of Water and Nitrogen Coupling on Photosynthetic Characteristics, Yield and Quality of Isatis Indigotica

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Abstract

In arid areas of Northwest China, *Isatis indigotica* is a commercial medicinal crop cultivated with higher water and nutrient in a large area, which makes rational irrigation and nitrogen application key factors for successful crop management. The objective of this study was to determine the effect of water and nitrogen coupling on photosynthetic characteristics, yield, and quality of *Isatis indigotica* produced in northwestern China. Field trials were conducted for two consecutive years on the irrigation test station. Data on photosynthetic parameters, yield and quality were collected from individual *Isatis indigotica* for each treatment during the years 2018–2019. The application of nitrogen significantly increased photosynthetic rates and yield under the same irrigation conditions. However, the yield were reduced in the excess water treatments, W3N1, and W3N2 and in excess nitrogen treatments, W1N3, W2N3, W3N3, in contrast to the optimum W2N2 treatment. Moreover, the quality indicators of the W2N2 treatment decreased compared with CK, which was due to water stress and more photo-assimilates being available to the roots, but the effective quality index value cloud be effectively improved by greatly increasing the yield.

1 Introduction

In the oasis region of Hexi, China, agricultural development is limited by water shortage and excessive application of nitrogen fertilizer. The main research question is how to improve water use efficiency and reduce nitrogen application. Two essential factors for crop growth are water and fertilizer. Rational irrigation and fertilization can effectively improve crop yield. The main problem facing modern agriculture is how to promote fertilizer with water and transfer water with fertilizer [1]. Reasonable water-nitrogen coupling optimization model has been paid more and more attention by researchers. Water is the medium through which soil nutrients are effectively absorbed by plants, improving the efficiency of nitrogen utilization. However, excessive water will lead to leaching and loss of nitrogen, while excessive nitrogen application will lead to non-point source pollution [2].

At present, more and more studies have been done in crops under the coupled conditions of water and nitrogen. For example, Liu et al[3]., Li et al[4]., Sui et al[5]., Fiasconaro et al[6]., Gholamhoseini et al[7].,respectively studied the coupling of water and nitrogen on the yield and quality of rice, tomato, cotton, alfalfa, corn and other crops. The synergistic effect of water and nitrogen can save water, increase yield and improve crop quality effectively. The yield and water use efficiency of *Isatis indigotica* were not high due to flood irrigation. At the same time, in order to increase yield, the phenomenon of excessive nitrogen application is very common [8].

Scholars have studied the effect of water on the yield and quality of *Isatis indigotica* from the aspects of water-saving irrigation system [9] and water stress[10]. Unfortunately, no research is currently available on the influence of water and nitrogen fertilizer on photosynthesis, yield and quality were not discussed. Local farmers fertilized and irrigated only relying on the empirical, resulted in low efficiency of water and fertilizer utilization. The present experiment was undertaken, therefore, to study the effect of water and nitrogen coupling on photosynthesis, yields and quality of *Isatis indigotica*.

2 Materials And Methods

2.1 Location

The experiments were carried out at Yimin Irrigation Pilot Station (Gansu, China; 100°43’E, 38°39’N) in the middle reaches of Flood River Irrigation District, Minle County, Gansu Province from May to October in 2018 and 2019. The permission for collecting of 'Isatis indigotica' were approved by Yimin Irrigation Experimental Station and Flood River Administration Office,Minle County，China. 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. 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 $g \cdot cm^{-3}$, groundwater level is low, and the area does not show salinization and alkalization.

2.2 Test Materials and Cultivation Methods

The full *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 $kg \cdot hm^{-2}$ and the planting density was 800,000 $plants \cdot hm^{-2}$. Before sowing, the experimental zone was ploughed for 30 cm to remove weeds manually. At the same time, 350 $kg \cdot hm^{-2}$ calcium superphosphate (12% of P_2O_5, 10% of S, 16 % of Ca) and 200 $kg \cdot hm^{-2}$ source potassium (25% of K_2O) was applied. Each experimental plot is separated by a film with a width of 60 cm to prevent water from seepage underground.

2.3 Experiment Design

The growth stage of *Isatis indigotica* is divided into four growth stages according to its growth characteristics: seedling stage, vegetative stage, fleshy root growth stage and fleshy root maturity. Three irrigation treatments were set in the field experiment, W1, W2, and W3, respectively, which were 60% ~ 70% of field water capacity, 70% ~ 80% of field water capacity, and 80% ~ 90% of field water capacity. Three nitrogen treatments, N1:150$kg \cdot hm^{-2}$, N2:200 $kg \cdot hm^{-2}$, N3:250 $kg \cdot hm^{-2}$.There are 10 water control treatments, of which CK was the control treatment. Each treatment was repeated three times, totaling 30 districts. The area of each district was 36 m^2 (9 m × 4 m). The method of irrigation is drip irrigation under mulch. The specific experimental design is shown in Table 1.

Table 1

Experimental treatment of nitrogen coupling in *Isatis indigotica*.

Treatment	Serial number	N application/(kg·hm^{-2})	Field water capacity
Low water Low nitrogen	W1N1	150	60%～70%
Low water Medium nitrogen	W1N2	200	60%～70%
Low water High nitrogen	W1N3	250	60%～70%
Medium water Low nitrogen	W2N1	150	70%～80%
Medium water Medium nitrogen	W2N2	200	70%～80%
Medium water High nitrogen	W2N3	250	70%～80%
High water Low nitrogen	W3N1	150	80%～90%
High water Medium nitrogen	W3N2	200	80%～90%
High water High nitrogen	W3N3	250	80%～90%
Control Treatment	W0N0	0	0

2.4 Photosynthetic characteristics

Photosynthesis was measured using a Li-6400 portable photosynthesis system (Zhangye, Gansu, China) during the period 9:30 – 10:30 a.m. on May 15, June 1, June 20, July 10, July 25, 2018 and 2019. The physiological parameters net photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate (Tr) and intercellular CO_2 concentration (Ci) were measured in situ for the seventh or eighth leaf that was fully expanded [11] (counted back from the apex of new shoots). In each treatment, three to four sunlit healthy leaves for each sampled plant were randomly selected from different directions and labeled; each leaf was measured once, in triplicate for each treatment. The average value of each treatment was calculated.

2.5 Yeild and Water use efficiency (WUE)

At harvest time, the yield of plot was weighed and counted separately, and the yield of each treatment was the average of three replicates.

$$WUE = Y/ET_a$$

where WUE is water use efficiency (kg·hm^{-2}·mm), Y is yield per unit area of *Isatis indigotica* (kg·hm^{-2}·mm), ET_a is water consumption (mm) during the whole growth period of *Isatis indigotica*.

2.6 Quality

Determination of indigo, indirubin, (R, S)-goitrin content: The method of Chinese Pharmacopoeia [12] was used to extract indigo, indirubin, (R, S)-goitrin, and its content was determined by high performance liquid chromatography. The content of polysaccharide in *Isatis indigotica* root was determined by phenol-sulfuric acid colorimetry.

2.7 Statistical analysis

The data analyses were performed using SPSS software (SPSS, Chicago, IL). The significance of differences between treatments for the different measured parameters was evaluated Independent samples followed by Kruskal-Wallis test ($P \leq 0.05$). The GraphPad Prism 5.01 was used to draw the graph. The data in each table were average values of three replicates.

3 Results And Discussion

3.1 Photosynthetic characteristics

Water and nitrogen coupling treatment had a significant effect on Photosynthetic characteristics (Fig. 1). Generally, the net photosynthetic rate in the treatment order: CK, W1N1, W1N3, W3N1, W3N3, W2N1, W1N2, W3N2, W2N3 and W2N2. The treatment with low water and low nitrogen was significantly lower than that with W2N2. The stomatal conductance and transpiration rate showed similar changing patterns. The net photosynthetic rate showed unimodal trend with the increase of nitrogen application at the same irrigation level. Under the same nitrogen application level, the net photosynthetic rate increased first and then decreased slowly with the increase of irrigation amount, showing the value of W2 > W3 > W1. The net photosynthetic rate was the highest and the mean value was 13.87 $\mu mol \cdot m^{-2} \cdot s^{-1}$ under treatment W2N2. The results showed that severe water stress and excessive nitrogen were not conducive to the absorption and utilization of water and nutrients by crop roots, which led to the decrease of photosynthetic rate. The effect of water and nitrogen treatment on intercellular CO_2 concentration was significant (Fig. 1). Under the condition of too much water or too much nitrogen, the photosynthesis of *Isatis indigotica* was disadvantageous, and the intercellular CO_2 concentration showed a trend opposite to the net photosynthetic rate.

Compared with the treatment of N, P and K deficiency, water-N coupling could increase the Pn of crops, which was the same as that of other fruit trees and vegetables [13]. 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 [14]. The net photosynthetic rate (Pn), stomatal conductance (GS) and transpiration rate (Tr) of maize leaves at seedling stage decreased significantly, while the intercellular CO_2 concentration (Ci) increased significantly when the nitrogen application rate was low [15].

The experiments with *Isatis indigotica* demonstrate that the net photosynthetic rate (Pn), stomatal conductance (GS) and transpiration rate (Tr) under the same irrigation level increased firstly and then decreased with the increase of nitrogen application rate. The net photosynthetic rate, transpiration rate and stomatal conductance of *Isatis indigotica* were improved by rational nitrogen application. Similar findings have been reported for *Isatis indigotica, w*ith the decreases of N level,the net photosynthetic rate transpiration rate and stomatal conductance of leaves gradually decreased while the intercellular CO_2 concentration (Ci) increased [16][17].

3.2 Yeild and Water use efficiency

The *Isatis indigotica* yield values presented are the average of two consecutive years of water nitrogen trials (Fig. 2). The *Isatis indigotica* yields differed significantly between the water nitrogen treatments; the W2N2 and W2N3 treatments had the highest yields of 7277.5 and 6820.5 $kg \cdot hm^{-2}$, respectively. The lowest yield of 3264.5 $kg \cdot hm^{-2}$ was recorded in the control treatment. The yield of all treatments was significantly higher than that of the control treatment. The yield of the W2N2 and W2N3 treatments was significantly higher than that of the W1N1 and the W3N1. With the increase of nitrogen application rate, the yield first increased and then decreased under the same irrigation condition.

The Water use efficiency values of *Isatis indigotica* presented are the average of two consecutive years of water nitrogen trials (Fig. 2). The Water use efficiency of *Isatis indigotica* differed significantly between the water nitrogen treatments; the W1N2 and W2N2 treatments had the highest yields of 20.78 and 19.63 $kg \cdot mm^{-1} \cdot hm^{-2}$, respectively. The lowest yield of 13.65 $kg \cdot mm^{-1} \cdot hm^{-2}$ was recorded in the W3N1 treatment. The Water use efficiency values of the W1N2 and W2N2 treatments was significantly higher than that of the W3N3, which was the treatment of excess water and nitrogen fertilizer. The Water use efficiency decreased with the increase of irrigation under the same nitrogen application condition. The Water use efficiency increased first and then decreased with the increase of nitrogen application rate under the same irrigation conditions. TheW2N2 treatment was the highest yield and Water use efficiency. Therefore, the water-nitrogen coupling mode of medium water medium nitrogen achieved the highest yield and effectively save water.

Generally, appropriate water deficit can improve crop yield and water use efficiency [18][19], and rational fertilization can increase crop yield, such as fruit trees and vegetables [20][21][22]. The yield increase in the current experiment was probably related to reasonable water stress and reasonable nitrogen application: theW2N2 treatment was the highest yield and Water use efficiency. However, too much water and too much nitrogen reduced yield and water use efficiency of the *Isatis indigotica*. This was consistent with recent research reports [23][24]. Compared with the local flooding irrigation and excessive nitrogen fertilizer mode, the W2N2 treatment with middle water and nitrogen not only obtained high yield, but also significantly improved the water use efficiency. It can also reduce the effect of excessive application of water and fertilizer on soil productivity, which is a better water and nitrogen management mode for local *Isatis indigotica* production.

3.3 Quality

The *Isatis indigotica* quality values presented are the average of two consecutive years of water nitrogen trials (Fig. 3). It mainly included the following content indicators: Indigo, Indirubin, (R, S)-goitrin and Polysaccharide. The *Isatis indigotica* quality indicators differed significantly between the water nitrogen treatments. The CK treatments had the highest values in all quality indicators, respectively. Each quality indicator decreased gradually with the increase of water content under the same nitrogen application conditions. Each quality indicator decreased gradually with the increase of nitrogen application under the same water condition. The content (R, S)-goitrin of the W2N2 treatment decreased by 6.5% compared with CK; decreased by 3.9% compared with W1N1 treatment.

Water is the medium for improving crop quality. Generally, the crop quality was improved by suitable water deficit [25][26][27] and reasonable fertilization [28][29][30]. Gradually, the quality of *Isatis indigotica* in the current experiment was accumulated with the decrease of water. The water deficit treatment increased the content of effective components and improved the quality of *Isatis indigotica*. The content of effective components in all treatments reached the pharmacopoeia standard [12]. The quality indicator values of each treatment in the current experiment were significantly lower than those of CK treatment, but there was little difference in the quality indicator values between each treatment. Moreover, the yield of the control treatment was much lower than other treatments. Therefore, the effective quality content of the control treatment is lower than other treatments. Too much water and nitrogen were not conducive to quality accumulation, which was not consistent with recent research reports [31]. That showed the total N applications over 280 kg ha^{-1} did not increase yield or quality and N above 56 kg ha^{-1} had no impact on plant biomass or tuber yield on any farm in any year of this study.

4 Conclusions

The current research indicates that there are some distinct benefits of water - nitrogen coupling. The W2N2 treatment of water and nitrogen in medium could significantly promote net photosynthetic rates, and increase the yield and water use efficiency, but lack or excess of water and nitrogen would greatly reduce the effects. Reasonable water stress had a significant positive impact on the quality of *Isatis indigotica* under the same nitrogen application condition, but had no significant effect on the yield. The yield and quality of *Isatis indigotica* could be improved by reasonable water stress and moderate nitrogen application.

Declarations

Acknowlegements

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Figures

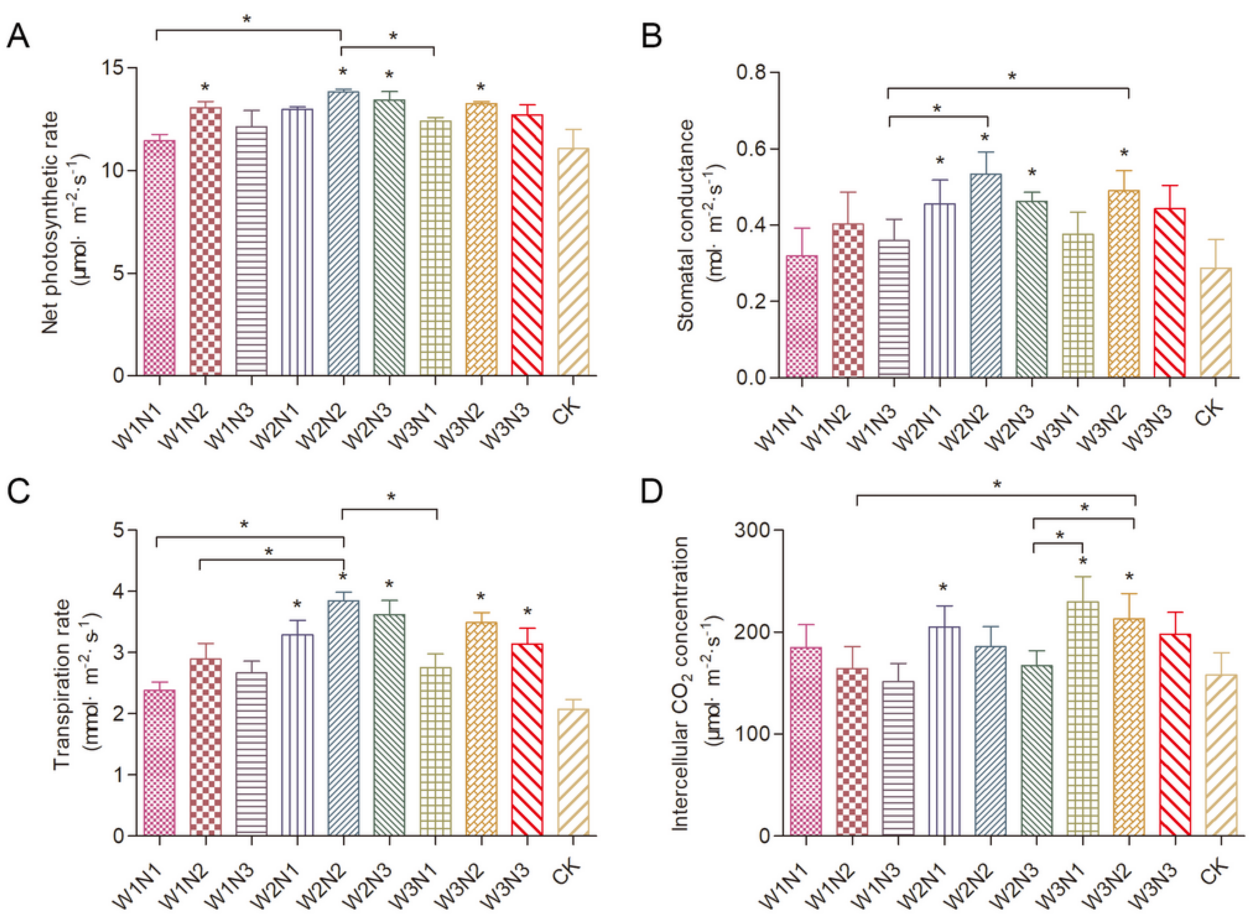

Figure 1

Photosynthetic characteristics values for Isatis indigotica in all treatments. Note: The values shown are the mean ± SD, n= 3. A marker with * indicates a significant difference at P ≤ 0.05 level

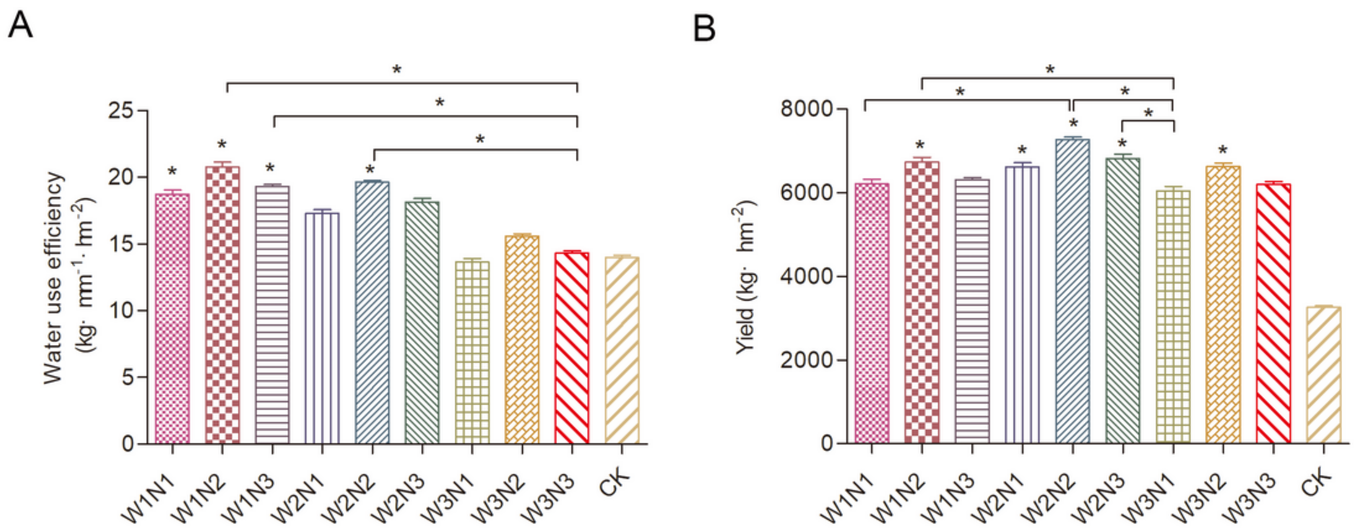

Figure 2

Yeild and Water use efficiency values for Isatis indigotica in all treatments. Note: The values shown are the mean ± SD, n= 3. A marker with * indicates a significant difference at P ≤ 0.05 level

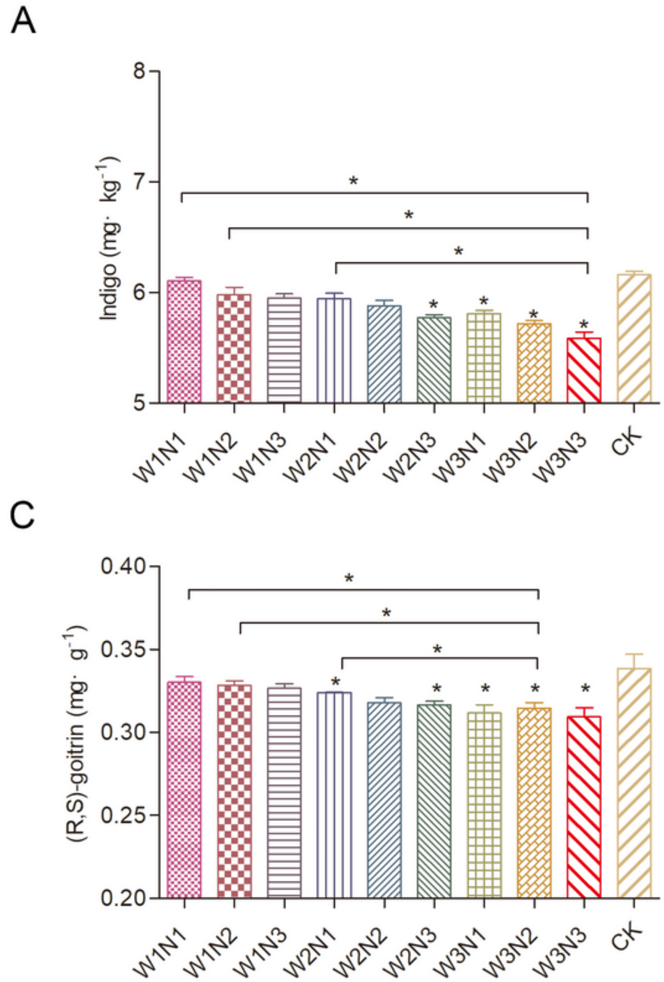

Figure 3

Quality values for Isatis indigotica in all treatments. Note: The values shown are the mean ± SD, n= 3. A marker with * indicates a significant difference at P ≤ 0.05 level