Effects of Water and Nitrogen Regulation on Nutrient Uptake, Water and Nitrogen Use Efficiency, and Yield of Baby Bok Choy: Postprint

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Abstract

Reasonable irrigation and nitrogen application rates are of great significance for improving nutrient use efficiency in pak choi and controlling non-point source pollution. This study employed a pot experiment using 15N isotopic tracer technique to investigate the effects of different irrigation levels (W1: 60% f; W2: 75% f; W3: 90% f, where f represents field capacity) and nitrogen application rates (N0: 0 $g \cdot kg^{-1}$; N1: 0.1 $g \cdot kg^{-1}$; N2: 0.2 $g \cdot kg^{-1}$; N3: 0.3 $g \cdot kg^{-1}$) on nutrient uptake, yield, and water-nitrogen use efficiency in pak choi. The results showed that both irrigation level and nitrogen application rate had significant effects on nitrogen, phosphorus, and potassium contents in roots and leaves of pak choi, and leaf phosphorus content was significantly affected by the water-nitrogen interaction. Leaf nitrogen and potassium contents were significantly greater than those in roots. With increased irrigation, phosphorus content and root nitrogen content in pak choi increased, while potassium content and leaf nitrogen content decreased; nitrogen application could increase nitrogen and potassium contents while decreasing phosphorus content. Irrigation and nitrogen application had significant effects on total nitrogen, phosphorus, and potassium uptake in pak choi, and phosphorus and potassium uptake were significantly influenced by the water-nitrogen interaction, with the medium water-low nitrogen treatment (W2N1) showing the maximum nutrient uptake. Pak choi yield was significantly affected by irrigation level and nitrogen application rate, showing an increase with rising irrigation levels and a trend of initial increase followed by decrease with increasing nitrogen rates. Irrigation water use efficiency (IWUE) was significantly affected by nitrogen application rate and water-nitrogen interaction, with IWUE changing consistently with yield as nitrogen application rate increased. Irrigation and nitrogen application had significant effects on 15N fertilizer fate, and fertilizer use efficiency was significantly influenced by the water-nitrogen interaction. With increasing irrigation

levels, fertilizer use efficiency showed an increasing trend, while the medium water treatment had the lowest fertilizer residual rate and the highest loss rate. With increasing nitrogen application rates, fertilizer use efficiency continuously decreased, while the loss rate showed an increasing trend. Under the conditions of this experiment, considering nutrient uptake, yield, and water-nitrogen use efficiency in pak choi comprehensively, the W3N1 and W2N1 combinations are recommended as optimal water-nitrogen treatments.

Full Text

Introduction

China leads the world in both area and production of greenhouse vegetables, making vegetable production a vital component of the national economy. However, unreasonable water and fertilizer management in production systems has led to reduced water and nutrient resource utilization efficiency, decreased product quality, and frequent problems such as nitrogen leaching losses and soil non-point source pollution. Water and nitrogen exhibit coupling effects that jointly influence crop growth, development, yield, and nitrogen use efficiency. Therefore, effective regulation of water and nitrogen supply in greenhouse vegetable production is crucial for exploiting synergistic effects. Numerous domestic and international scholars have conducted extensive research on how water and nitrogen affect nutrient uptake, yield, and water/nitrogen use efficiency in crops. Studies have shown that appropriately increasing nitrogen application and irrigation rates benefits the absorption of nitrogen, phosphorus, and potassium, with nutrient uptake showing significant positive correlations with crop dry matter accumulation and yield. Research by Cai et al. on greenhouse vegetables under optimized water-fertilizer coupling conditions demonstrated that nitrogen, phosphorus, and potassium content, absorption rates, and accumulation in vigorously growing vegetables were significantly greater than in seedlings and mature plants. Both irrigation and nitrogen application rates significantly affect crop production and yield, with yield positively correlated with irrigation amount within a certain range and showing a parabolic relationship with nitrogen rate. Li et al. found that cucumber dry matter accumulation, irrigation water use efficiency, and water use efficiency were significantly or extremely significantly affected by nitrogen rate, irrigation amount, and their interaction (coupling effect). Xiao et al. reported that lettuce total nitrogen absorption increased initially then decreased with increasing nitrogen application, while nitrogen use efficiency gradually decreased and nitrogen loss rate continuously increased.

Rape (*Brassica campestris*), a representative leafy vegetable with high nutritional value and multiple cropping index, has received limited comprehensive research on how water and nitrogen regulation affects its nitrogen, phosphorus, and potassium uptake and water/nitrogen use efficiency. The underlying water-nitrogen coupling mechanism remains unclear. The ^{15}N tracer technique serves as an important tool for studying fertilizer utilization efficiency and fate. Therefore, this experiment used rape as test material and employed ^{15}N tracer technology to investigate the effects of different irrigation and nitrogen application rates on nutrient uptake, yield, and water/nitrogen use efficiency, aiming to provide a theoretical basis for efficient rape production.

Materials and Methods

1.1 Experimental Site and Materials

The experiment was conducted in September 2014 in a solar greenhouse at the Shenyang Agricultural University experimental base. Pot cultivation experiments were performed using pots with an upper inner diameter of 24 cm, bottom inner diameter of 18 cm, and height of 17 cm. Each pot contained 4 kg of air-dried soil passed through a 3 mm sieve. The test soil was brown earth collected from open vegetable fields, with the following properties: organic matter 19.02 $g \cdot kg^{-1}$, pH 5.95, total nitrogen 0.97 $g \cdot kg^{-1}$, total phosphorus 0.41 $g \cdot kg^{-1}$, total potassium 22.24 $g \cdot kg^{-1}$, alkaline hydrolysis nitrogen 83.07 $mg \cdot kg^{-1}$, available phosphorus 5.23 $mg \cdot kg^{-1}$, available potassium 80.36 $mg \cdot kg^{-1}$, and field capacity of 30.5%. The test rape variety was 'Qingbang Chinese cabbage type'.

1.2 Experimental Design and Management

The experiment employed a completely randomized design with three irrigation levels [W1: 60%θ_f (low water), W2: 75%θ_f (medium water), and W3: 90%θ_f (high water), where θ_f represents field capacity] and four nitrogen rates [N0: 0 $g \cdot kg^{-1}$ (no nitrogen), N1: 0.1 $g \cdot kg^{-1}$ (low nitrogen), N2: 0.2 $g \cdot kg^{-1}$ (medium nitrogen), and N3: 0.3 $g \cdot kg^{-1}$ (conventional nitrogen rate)], totaling 12 treatments with three replicates. All treatments received identical phosphorus and potassium application rates, which were thoroughly mixed with the soil as base fertilizers. Phosphorus fertilizer was applied as calcium superphosphate at 0.2 $g(P_2O_5) \cdot kg^{-1}$, and potassium fertilizer as potassium sulfate at 0.3 $g(K_2O) \cdot kg^{-1}$. The nitrogen fertilizer used was stable isotope ^{15}N-labeled urea (20.14% abundance, Shanghai Chemical Industry Research Institute), which was uniformly applied to the soil in aqueous solution three days before sowing. Rape was sown on September 10, and after full emergence on September 17, seedlings were promptly thinned to eight plants per pot after true leaf development. Irrigation amounts were consistent across treatments before seedling establishment. After establishment, soil relative water content was controlled using the weighing method, with soil water content adjusted once daily and irrigation amounts recorded.

1.3 Measurements and Methods

Plants were harvested at the 4-leaf stage (28 days after sowing) and 6-leaf stage (40 days after sowing), with three and five plants collected, respectively. Harvested plants were immediately separated into roots and leaves, placed in selfsealing bags, and transported to the laboratory. After washing with clean water and rinsing twice with distilled water, samples were quickly dried with gauze and weighed by pot to determine yield, expressed as total fresh weight Y (g·pot^{-1}) from both harvests. After yield determination, samples were deactivated at 105 °C for 30 minutes, then dried and ground at 60 °C.

Total nitrogen and ^{15}N atom percent excess were determined using an elemental analyzer (VARIO EL III, Germany) coupled with a stable isotope ratio mass spectrometer (Isoprime 100, England). Plant phosphorus and potassium contents were measured after H_2SO_4-H_2O_2 digestion using the molybdenum-antimony anti-colorimetric method and atomic absorption method, respectively.

Irrigation water use efficiency (IWUE) was calculated as:

$$\text{IWUE } (\text{g} \cdot \text{L}^{-1}) = \frac{\text{Yield } (\text{g} \cdot \text{pot}^{-1})}{\text{Irrigation amount } (\text{L} \cdot \text{pot}^{-1})} \tag{1}$$

Total nitrogen uptake (mg·pot^{-1}) = [Total leaf dry weight (mg·pot^{-1}) × leaf N%] + [Total root dry weight (mg·pot^{-1}) × root N%]

The fate of ^{15}N fertilizer, including ^{15}N fertilizer use efficiency (%), ^{15}N fertilizer residual rate (%), and ^{15}N fertilizer loss rate (%), was calculated using the following formulas [20-21]:

$$^{15}\text{N fertilizer use efficiency } (\%) = \frac{\text{Plant N uptake } (\text{mg} \cdot \text{pot}^{-1}) \times {}^{15}\text{N atom percent excess in plant}}{\text{N application rate} \times {}^{15}\text{N atom percent excess in fertilizer}} \times 100 \tag{3}$$

$$^{15}\text{N fertilizer residual rate } (\%) = \frac{\text{Soil dry weight} \times \text{Soil N\%} \times \text{Soil } {}^{15}\text{N atom percent excess}}{\text{N application rate} \times {}^{15}\text{N atom percent excess in fertilizer}} \times 100 \tag{4}$$

$$^{15}\text{N fertilizer loss rate } (\%) = 100\% - {}^{15}\text{N fertilizer use efficiency } (\%) - {}^{15}\text{N fertilizer residual rate } (\%) \tag{5}$$

Statistical analysis was performed using SPSS 21 software, with Duncan's method employed for multiple comparisons.

Results

2.1 Effects of Water and Nitrogen Regulation on Nitrogen Uptake in Rape

Two-way ANOVA indicated that both leaf and root nitrogen contents were significantly affected by irrigation level and nitrogen rate individually, with leaf nitrogen content significantly greater than root nitrogen content . At the W1 irrigation level, leaf nitrogen content was significantly higher than at W2 and W3, while no significant difference existed between W2 and W3. Root nitrogen content under W3 treatment was significantly higher than the other two treatments, with no significant difference between W1 and W2. Nitrogen application significantly increased leaf and root nitrogen contents of rape, though no significant differences were observed among nitrogen treatments.

Nitrogen rate had significant effects on leaf, root, and whole-plant nitrogen uptake, all showing the pattern N1, N2 > N3 > N0, indicating that appropriate nitrogen application benefited rape nitrogen uptake. Irrigation level only significantly affected root nitrogen uptake, showing the pattern W3, W2 > W1.

2.2 Effects of Water and Nitrogen Regulation on Phosphorus Uptake in Rape

As shown in , both irrigation amount and nitrogen rate significantly affected leaf and root phosphorus contents, with leaf phosphorus content significantly influenced by the water-nitrogen interaction. Leaf and root phosphorus contents decreased with increasing irrigation level. The no-nitrogen treatment had significantly higher leaf phosphorus content than nitrogen treatments, while no significant differences existed among nitrogen treatments. The N3 treatment showed the maximum root phosphorus content, while N2 showed the minimum, with a significant difference between them. Under water-nitrogen interaction, leaf phosphorus contents among nitrogen treatments differed significantly at the W3 irrigation level, while no significant differences were observed at W1 and W2 irrigation levels.

Two-way ANOVA revealed that irrigation level, nitrogen rate, and their interaction all significantly affected whole-plant and leaf phosphorus uptake, with effects in the order: water > water-nitrogen interaction > nitrogen rate. The effect of irrigation on phosphorus uptake followed the pattern W2 > W1 > W3. The N1 and N2 treatments showed the maximum phosphorus uptake with no significant difference between them, but both were significantly greater than N3. Under water-nitrogen interaction, the W2N1 treatment showed the maximum phosphorus uptake, followed by W1N2, while W3N1 and W3N3 showed the minimum.

2.3 Effects of Water and Nitrogen Regulation on Potassium Uptake in Rape

As shown in , leaf potassium content was approximately twice that of roots and was significantly affected by both irrigation level and nitrogen rate. The W1 treatment showed significantly greater leaf and root potassium contents than W2 and W3, with no significant difference between W2 and W3, indicating that lower irrigation rates resulted in higher potassium content in rape. With increasing nitrogen application rate, both leaf and root potassium contents showed an increasing trend.

Two-way ANOVA indicated that leaf, root, and whole-plant potassium uptake were significantly affected by nitrogen rate and water-nitrogen interaction, while irrigation level alone had no significant effect. The effect of nitrogen rate on leaf and whole-plant potassium uptake followed the pattern N1 > N2 > N3 > N0, while root potassium uptake showed the pattern N2 > N1 > N0 > N3, suggesting that appropriate nitrogen application benefited rape potassium accumulation. Under water-nitrogen interaction, the W2N1 treatment showed the maximum leaf and whole-plant potassium uptake, significantly greater than other treatments, while W2N2 showed the maximum root potassium uptake.

2.4 Effects of Water and Nitrogen Regulation on Yield, Irrigation Water Use Efficiency, and Fertilizer Fate

Variance analysis revealed that both irrigation level and nitrogen rate had extremely significant effects on rape yield, while their interaction did not . Rape yield increased with irrigation level, with no significant difference between W2 and W3, but showed a parabolic trend with nitrogen rate, with no significant difference between N1 and N2.

Nitrogen rate had an extremely significant effect on irrigation water use efficiency (IWUE), with a significant interaction effect also observed . IWUE showed a parabolic trend with nitrogen rate, with no significant difference between N1 and N2. Under water-nitrogen interaction, the W1N1 treatment showed the maximum IWUE, which was not significantly different from W3N2, W1N2, W2N1, W2N2, W3N1, or W2N3 treatments. The W3N3 treatment showed the minimum IWUE, followed by W1N0 and W2N0 treatments.

Research on the fate of ^{15}N fertilizer under different water-nitrogen treatments showed that both water and nitrogen significantly affected ^{15}N fertilizer utilization. With increasing irrigation level, ^{15}N fertilizer use efficiency showed an increasing trend, while the W2 treatment showed the lowest fertilizer residual rate and highest loss rate. With increasing nitrogen rate, fertilizer use efficiency continuously decreased while loss rate showed an increasing trend, with residual rate showing no significant change. Water-nitrogen interaction significantly affected fertilizer use efficiency. The W3N1 treatment showed the maximum nitrogen use efficiency at 23.00%, followed by W2N1 at 18.41%, while W1N3 showed the minimum at only 5.30%. The W3N1 treatment had the highest fertilizer use efficiency, lowest loss rate, and moderate residual rate, making it the optimal water-nitrogen treatment considering fertilizer utilization. Additionally, the W2N1 treatment showed relatively high fertilizer use efficiency with low residual and loss rates .

Nutrient transport between plant leaves and roots depends on concentration gradients within the plant, resulting in significant correlations between root and leaf nutrient contents that vary by growth stage. In this experiment, leaf nitrogen and potassium contents were significantly greater than root contents because rape leaves had vigorous photosynthetic activity at harvest, requiring substantial nutrient supply and thus transferring more nutrients from roots to leaves. Furthermore, this experiment showed that nutrient accumulation under different water-nitrogen treatments was not significantly correlated with nutrient content but was positively correlated with yield (except for phosphorus uptake), consistent with previous research. Nitrogen application can increase plant nitrogen, phosphorus, and potassium uptake, but excessive nitrogen reduces uptake, as verified in rice (*Oryza sativa*), maize (*Zea mays*), melon (*Cucumis melo*), and cotton (*Gossypium sp.*). In this experiment, when nitrogen application exceeded 0.1 g·kg^{-1} soil, rape nitrogen, phosphorus, and potassium uptake showed no significant increase or even a decreasing trend. This occurs because excessive nitrogen produces toxic effects that inhibit normal plant growth and nutrient absorption. Irrigation level also significantly affected phosphorus uptake, with W2 showing maximum phosphorus absorption but no significant effect on nitrogen and potassium uptake. Additionally, water-nitrogen coupling significantly affected phosphorus and potassium accumulation. Rational regulation of water and nitrogen supply improved rape nutrient uptake and accumulation, with the W2N1 treatment (75%θ_f, 0.1 g·kg^{-1}) showing maximum nitrogen, phosphorus, and potassium uptake.

Rape yield is closely related to water-nitrogen supply and water-nitrogen use efficiency. In this experiment, yield increased with irrigation level and showed a parabolic trend with nitrogen rate, consistent with Badr et al. Additionally, yield was positively correlated with IWUE and nitrogen use efficiency ($P < 0.05$, data not shown), as higher water and nutrient uptake provides sufficient raw materials for leaf photosynthesis, increasing photosynthetic products and promoting rape yield.

Both irrigation and nitrogen rate can affect crop IWUE, with research indicating IWUE is significantly negatively correlated with irrigation amount and nitrogen application can improve IWUE. In this experiment, IWUE showed no significant difference among irrigation levels, while nitrogen rate had an extremely significant effect. This may occur because low irrigation creates water stress that inhibits rape biomass growth, resulting in lower IWUE. Appropriately increasing irrigation ensures adequate water for rape growth, maintains proper soil porosity, reduces soil surface evaporation, and improves irrigation water use efficiency, consistent with findings by Deng et al. and Cabello et al. In this experiment, IWUE showed a parabolic trend with nitrogen rate, with regression analysis indicating maximum IWUE at a nitrogen rate of 0.16 g·kg^{-1}.

In greenhouse vegetable fields, applied fertilizers remain mostly in the soil after crop absorption and losses, posing potential environmental threats. In this experiment, ^{15}N fertilizer use efficiency ranged from 5.30% to 23.00%, residual rate from 56.22% to 71.29%, and loss rate from 13.17% to 37.10% under different water-nitrogen treatments, similar to results from greenhouse and open-field vegetable studies but lower than cereal crops. Lower fertilizer use efficiency in vegetables occurs because vegetable plants are smaller with lower fertilizer absorption than cereals, yet actual application rates are higher. Additionally, this experiment used one-time basal fertilizer application, resulting in lower use efficiency than multiple topdressing applications. In this experiment, rape ^{15}N fertilizer use efficiency increased with irrigation level and decreased with nitrogen rate, consistent with previous research.

In summary, appropriately increasing irrigation and applying suitable nitrogen rates can increase rape nitrogen, phosphorus, and potassium uptake and improve yield and water-nitrogen use efficiency. Under the conditions of this experiment, W3N1 and W2N1 are recommended water-nitrogen treatments. Although pot experiments differ from actual production, these results provide guidance for further field experiments and production management.

Conclusion

Different irrigation levels, nitrogen rates, and their interactions had varying effects on rape nitrogen, phosphorus, and potassium contents and uptake. The W2N1 treatment ($75\%\theta_f$, 0.1 $g \cdot kg^{-1}$) showed maximum nitrogen, phosphorus, and potassium uptake. Rape yield was significantly affected by irrigation level and nitrogen rate, increasing with irrigation level and showing a parabolic trend with nitrogen rate. Irrigation water use efficiency (IWUE) was significantly affected by nitrogen rate and water-nitrogen interaction, with IWUE changes following the same pattern as yield changes with increasing nitrogen rate. ^{15}N fertilizer use efficiency decreased with nitrogen rate and increased with irrigation level, with the W3N1 treatment ($90\%\theta_f$, 0.1 $g \cdot kg^{-1}$) showing the highest ^{15}N use efficiency and lowest loss rate. Considering rape nutrient uptake, yield, and fertilizer fate comprehensively, W3N1 and W2N1 are recommended as optimal water-nitrogen treatments.

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Note: Figure translations are in progress. See original paper for figures.