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Growth Characteristics, Water and Nitrogen Use Efficiencies of Spinach in Different Water and Nitrogen Levels

(Ciri Pertumbuhan, Air dan Kecekapan Penggunaan Nitrogen Bayam dalam Tahap Air dan Nitrogen yang Berbeza)

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ABSTRACT

Water deficit and environmental pollution owing to excessive nitrogen use have caused considerable attention. In a field experiment, a combination of three water levels (20, 40 and 60 cm) and nitrogen fertilizer rates (0, 85 and 170 kg ha^{-1}) was applied. The main objectives of this study were to optimize water and nitrogen application and exploit their interactive effects on the growth characteristics, yield and water and nitrogen use efficiency of spinach. The results showed that water and nitrogen significantly influenced average plant height and leaf area. Total aboveground biomass (TB) was affected by nitrogen fertilizer and TB decreased in water deficit. Adding nitrogen fertilizer amount resulted in higher leaf chlorophyll content and chlorophyll content obtained the maximum value in N_2 treatment, but chlorophyll content was not affected by water deficit. Spinach yield was higher at N_1 compared with N_0 and N_2 at all water levels. Abundant water supply resulted in the highest spinach yield, but yield reduced at lower water level (W_3). The correlation analysis between spinach yield and leaf number was relatively weak (R^2=0.58). On the contrast, the correlation analysis between spinach yield and leaf weight showed a correlationship (R^2=0.91), indicating that leaf weight was the primary reason for yield increase in all treatments. Nitrogen fertilization significantly decreased NUE in all the treatments. WUE of spinach increased with adding nitrogen application in most conditions.

Keywords: Biomass; growth; nitrogen use efficiency; water; yield

ABSTRAK

Defisit air dan pencemaran alam sekitar yang disebabkan oleh penggunaan nitrogen secara berlebihan telah mendapat perhatian umum. Dalam kajian yang dijalankan, gabungan tiga peringkat air (20, 40 dan 60 cm) dan kadar baja nitrogen (0, 85 dan 170 kg ha^{-1}) telah digunakan. Objektif utama kajian ini adalah untuk mengoptimumkan aplikasi air dan nitrogen dan mengeksploitasi kesan interaktif ciri-ciri pertumbuhan, hasil serta air dan nitrogen menggunakan kecekapan bayam. Hasil kajian menunjukkan bahawa air dan nitrogen ketara mempengaruhi purata ketinggian pokok dan luas daun. Jumlah biojisim atas permukaan tanah (TB) terjejas akibat baja nitrogen dan TB menurun dalam defisit air. Tambahan jumlah baja nitrogen telah meningkatkan kandungan klorofil daun yang tinggi dan kandungan klorofil telah mencapai nilai maksimum dalam rawatan N_2 tetapi kandungan klorofil tidak terjejas akibat kekurangan air. Hasil bayam adalah lebih tinggi pada N_1 berbanding N_0 dan N_2 dalam semua peringkat air. Bekalan air yang banyak telah menghasilkan jumlah bayam yang tinggi, tetapi hasil berkurangan pada tahap air yang lebih rendah (W_3). Analisis korelasi antara hasil bayam dan jumlah daun adalah agak lemah (R^2= 0.58). Sebaliknya, analisis korelasi antara hasil bayam dan berat daun menunjukkan hubungan korelasi (R^2= 0.91) yang menunjukkan bahawa berat daun adalah sebab utama peningkatan hasil dalam semua rawatan. Pembajaan nitrogen ketara menurunkan NUE dalam semua rawatan. WUE bayam meningkat dengan penambahan aplikasi nitrogen dalam kebanyakan keadaan.

Kata kunci: Air; biojisim; hasil; kecekapan penggunaan nitrogen; pertumbuhan

INTRODUCTION

Water is significant in plant nutrition absorption and transport process (Patanè & Cosentino 2010; Vandoorne et al. 2012). Many plants need water in order to get good quality and high yield. However, water deficit is often an important environmental factor limiting plant growth, resource allocation patterns and survival and many countries are subject to prolonged drought (Li et al. 2010). The first effect of water deficit is reducing leaf number (Golombek & Al-Ramamneh 2002) and leaf area of every plant (Nagaz et al. 2009) and then yield and dry matter production (Wang et al. 2006). Many researchers found water deficit reduced the leaf area (Pandey et al. 2000), plant height (Soler et al. 2007), shoot growth (Stone et al. 2000) and plant yield (Payero et al. 2006). Consequently, it is necessary to improve water use efficiency in agriculture and develop an efficient irrigation management strategy.

Nitrogen nutrition has an important role in both plant growth and development (Meyer & Marcum 1998;

Waddell et al. 1999). But farmers do not consider the response of different plants to nitrogen rate and forms and have gradually increased usage of nitrogen fertilizers (Wang et al. 2006). Adequate supply of nitrogen fertilizer is beneficial for the growth and development of most plants (Collins & McCoy 1997), but inappropriate practice makes noxious compounds accumulate in the edible products. Several studies showed that the application of more nitrogen fertilizer, plant yield and nitrogen concentration could increase, but nitrogen use efficiency might decrease (Seghatoleslami et al. 2008; Sun et al. 2009).

Spinach is the most important leafy vegetable and has a production area of 8.9×10^4 ha area and amount of 14.04 million tons in the world in 2007 (Anonymous 2008). Spinach is characterized by high nutritive values and has a high content of dietary fibre and vitamins as well as mineral components such as magnesium and calcium. Spinach (*Spinacia oleracea* L.) is highly responsive to nitrogen fertilization (Cantliffe 1972) and is easy to accumulate nitrate because of an efficient uptake system and inefficient reductive systems. Nitrate is harmful to humans and may possibly make a contribution to surface and ground water pollution through leaching and soil erosion (Wang et al. 2006; Zhang et al. 2012). Water and fertilizer are two key factors influencing spinach yield and quality in agricultural production system.

Nitrogen competition of plants has been identified by several authors and water competition should also occur (Celette & Gary 2013). There have been many researches done on the effects of water and nitrogen on potato, wheat, rice and tomato in the world (Achten et al. 2010; Estrada-Campuzano et al. 2012; Onder et al. 2005; Patanè 2011; Sun et al. 2012). However, there are limited studies on evaluation of spinach production ability. At the same time, reports have seldom been performed on water and nitrogen use efficiency of spinach under different water and nitrogen levels. Therefore, evaluating the vegetables production response of spinach to irrigation in combination with nitrogen fertilizer could help to manage the resources. The effective management to enhance economical returns by applying less water and nitrogen is a main goal in China area. The aim of this study was to determine the interactive effect of water and nitrogen at different levels on spinach yield and growth parameters and water and nitrogen use efficiency.

MATERIALS AND METHODS

LOCATION AND GROWTH CONDITIONS

The field experiment was conducted at Irrigation and Drainage Experimental Station (E121°1', N31°1'), which is located in Qingpu District, Shanghai, China. Spinach was planted on 18 December, 2012 and harvested on 13 March, 2013. The soil of the experimental site was silty loam (according to international textural grade) in texture, composing 13.10% clay, 59.86% silt and 27.05% sand, with pH of 6.16, EC of 0.37 ms/cm and 1.18% organic matter in the upper 0-20 cm soil layer. The climatic parameters were taken from the automatic weather station in Qingpu Irrigation and Drainage Experimental Station, Shanghai. The detailed climatic parameters recorded from December in 2012 to March in 2013 during the growth season were summarized in Table 1.

EXPERIMENTAL DESIGN AND TREATMENTS

The experiment was arranged based on randomized block factorial design consisting of combinations of three irrigation treatments (W), as the main factor and three nitrogen levels (N). Irrigation water treatments includes 20 cm (W_1), 40 cm (W_2) and 60 cm (W_3) water levels and the amounts of nitrogen fertilizer were 0 kg ha^{-1}, 85 kg ha^{-1} and 170 kg ha^{-1}, respectively. The plot size was 2 × 3.33 m and treatments were designed with three replicates. Nitrogen fertilizer was also dissolve in the irrigation water and applied with irrigation water at a time when spinach had five main leaves.

Irrigation treatments This experiment used automatic water supply system which consisted of water tank, automatic water supply and drainage equipment, pipe and pipe network. There were three set water levels in this study. When water of plot was lack, water in the tank could be controlled by water supply device and went through the pipe to pipe network into the plot soil. While water level returned to the set level, water supply stopped. Water levels were set when spinach had five main leaves. Time Domain Reflectometry (TDR) probes were installed to monitor soil water level and calculate the irrigation amount needed in each treatment. For this research, nine treatments were presented in Table 2.

TABLE 1. Average monthly maximum (T_{max}) and minimum (T_{min}) temperature, relative humidity and evapotranspiration (ETo) during the season

Month	T_{max} (ºC)	T_{min} (ºC)	Relative humidity (%)	ETo (mm)
December	10.5	-5.0	81.2	1.1
January	17.0	-5.0	79.2	0.9
February	16.7	-4.0	81.8	0.9
March	31.0	-0.2	68.2	2.3

TABLE 2. Water content and nitrogen-fertilizer amounts under different treatments applied in the study

Experimental treatments	Water level (cm)	Nitrogen fertilizer (kg ha^{-1} (urea))
W_1N_0	20	0
W_1N_1	20	85
W_1N_2	20	170
W_2N_0	40	0
W_2N_1	40	85
W_2N_2	40	170
W_3N_0	60	0
W_3N_1	60	85
W_3N_2	60	170

MEASUREMENTS OF SPINACH PARAMETERS

Dry matter accumulation was assessed through harvesting three typical plants per replicate of each treatment. Samples were separated to leaves, stems and roots, dried at 70°C for 48 h in a forced air oven. Then dry weight was weighted and total aboveground biomass (TB) was calculated. Chlorophyll was measured with Dualex 4. Leaf chlorophyll of the youngest expanded leaf of 20 plants per replicate and leaf's midrib was avoided.

Water use efficiency (WUE) (Badr et al. 2012) was calculated from the spinach yield (kg ha^{-1}) divided by spinach water used for each water treatment in the growth period and unit was kg yield^{-1}mm^{-1}. Nitrogen use efficiency (NUE) was calculated using the following equation:

$$NUE = \frac{Yt - Yo}{N}.$$

Note: Yt is yield in each treatment, Yo is yield in control and N is used nitrogen. Unit is kg ha^{-1}.

STATISTICAL ANALYSIS

Mean comparison was carried out using least significant differences (LSD) test through statistical software SPSS 15.0 (SPSS Inc., Chicago, IL). In cases where the Tukey showed significant differences among means, the differences among treatments were compared using least significant differences (LSD) test at 5% significance level. All figures were analyzed by SIGMAPLOT 10.0 (Systat Software Inc., Chicago, USA).

RESULTS AND DISCUSSION

GROWTH CHARACTERISTICS AND BIOMASS MEASUREMENT

Water and nitrogen significantly influenced average plant height and leaf area. Plant height and leaf area increased with nitrogen application in the well-watered treatment, but excessive nitrogen inhibited their growth. In the water deficit treatment (60 cm), nitrogen application increased plant height and leaf area (Figure 1), but the increase was less than that of the abundant water treatment (20, 40 cm). Nishihara et al. (2001) reported that when soil water matric heads was -20 to -30 cm or -30 to -40 cm, the height was significantly greater than that in (-10 to -20 cm) treatment. Leaf area of spinach was consistent with that of height. Photosynthesis was restrained firstly due to stomatal closure under water deficit condition. Furthermore, water deficit might expedite senescence gradient of leaf.

Total aboveground biomass was affected by nitrogen fertilizer (p<0.05) (Figure 2(a). Under higher nitrogen treatment (N_1), the total aboveground biomass of spinach was higher than that of the contrast nitrogen treatment (N_0). Biomass increased 0.7 g (W_2N_1) and 0.9g (W_3N_1), respectively. But excessive nitrogen fertilizer could reduce plant growth and biomass (N_2). The difference of biomass was greater between high treatment (N_1) and contrast treatment (N_0). Water deficit had a negative effect on the total aboveground biomass in all nitrogen treatments (p<0.05). However, there was no significant difference in total aboveground biomass between the over-water and appropriate irrigation (W_1 and W_2).

Total aboveground biomass decreased in response to water deficit. These results were similar to earlier reports of Nishihara et al. (2001), who found that water deficit restrained spinach biomass. Poorter and Nagel (2000) indicated water and nutrient limitations led to carbon translocate from leaves to roots and as a result of reducing total aboveground biomass. Our investigation was also similar to these findings.

Leaf chlorophyll content of spinach was influenced by nitrogen application (Figure 2(b)). Adding nitrogen fertilizer resulted in higher leaf chlorophyll content and chlorophyll content obtained the maximum value in N_2 treatment. In contrast, chlorophyll content was not affected by water deficit. Wu et al. (2008) proposed that nitrogen deposition was likely to lessen slight water deficit.

YIELD

Application of nitrogen fertilizer had a significant effect on spinach yield (Table 3). There was a great difference among N_1 and N_0 versus N_2. Spinach yield was higher at

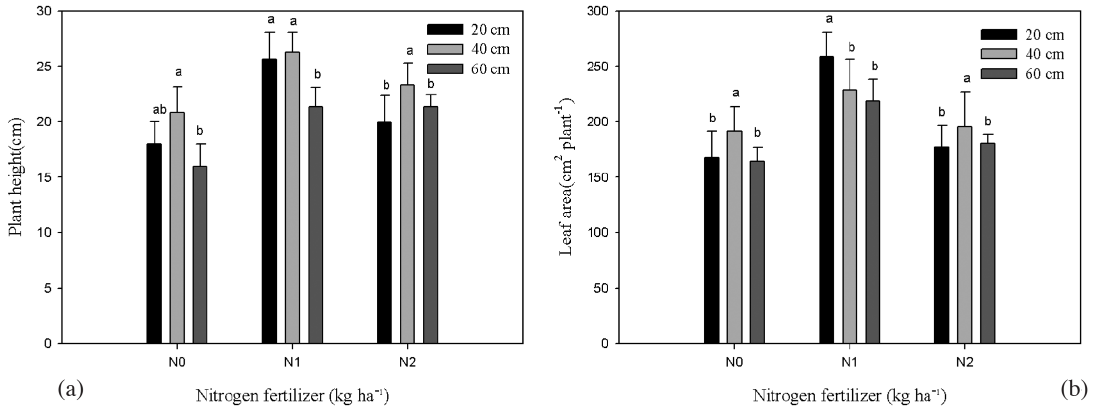

FIGURE 1. Plant height (a) and leaf area (b) of spinach at different water and nitrogen levels. Bars represent means of 10 replicates±standard deviations. Within each treatment, values accompanied by different letters differ significantly at $p<0.05$

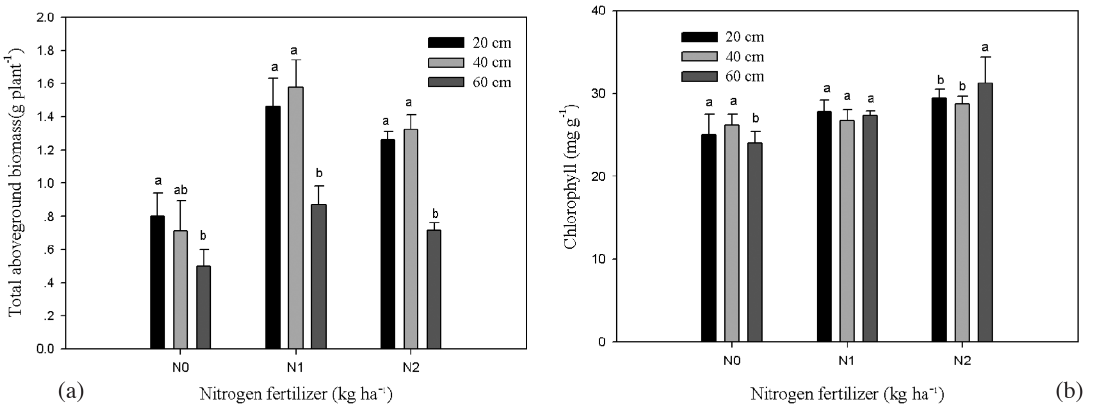

FIGURE 2. Total aboveground biomass (a) and chlorophyll content (b) of spinach at different water and nitrogen levels. Bars represent means of 10 replicates±standard deviations. Within each treatment, values accompanied by different letters differ significantly at $p<0.05$

N_1 compared with N_0 and N_2 at all water levels. Abundant water supply resulted in the highest spinach yield under all nitrogen fertilizer amounts, but yield reduced at lower water level (W_3). Darwish et al. (2003) and Patel and Rajput (2007) found similar results.

Leaf number and leaf weight of each plant decreased under water deficit in all nitrogen fertilizer amounts (Table 3). These trends were likely be the yield trend. Leaf weight was less resistant to drought than leaf number. Therefore, the decrease of spinach yield under drought treatment was primarily due to the reduction of leaf weight.

The correlation analysis between spinach yield and leaf weight showed a correlationship (R^2=0.91), indicating that leaf weight was the primary reason for yield increase in all treatments (Figure 3(a)). On the other hand, the correlation analysis between spinach yield and leaf number was relatively weak (R^2=0.58), which indicated that the decrease of leaf number does not bring about the reduction of spinach yield (Figure 3(b)). The combined effect between water and nitrogen for yield, leaf number and leaf weight was significant. This showed that both factors did not work, respectively. Spinach obtained the highest yield at W_2N_1, but yield only reached the least value at W_3N_2 as compared with the contrast treatment. These results showed that excessive nitrogen fertilizer had a contrary effect on spinach yield in water deficit condition. Thus, nitrogen application could get the highest yield under sufficient water and yield decreased under drought.

WATER AND NITROGEN USE EFFICIENCY

Different water levels and nitrogen fertilizer amounts both significantly affected nitrogen use efficiency. Effects on the NUE, the water levels ranked as $W_1 > W_2 > W_3$. NUE obtained the maximum value 62.47 kg yield kg^{-1}N in W_1 treatment and the minimum value was 6.53 kg yield kg^{-1}N in the lowest water level W_3 (Table 4). This indicated that abundant irrigation supply could accelerate NUE of spinach, but water deficit could reduce NUE. However, the optimum nitrogen level of the NUE was observed at N_1 level between W_1 and W_2 level and the highest NUE was observed at N_1 level.

Compared with the N_0 treatment, WUE of spinach increased with increasing nitrogen application in most

TABLE 3. Yield, leaf number, leaf weight of each plant and plant yield of spinach in different water and nitrogen treatment. Data in the table are means (n=3), comparison within the same column was performed with Tukey test. Rates of irrigation and nitrogen fertilization are cm and kg ha^{-1}, respectively

Nitrogen application	Irrigation level	Yield (ton ha^{-1})	Leaf number (plant^{-1})	Leaf weight (g plant^{-1})	Plant yield (g plant^{-1})
N_0	W_1	6.75b	6.67a	6.19b	7.41b
	W_2	8.76a	7.67a	7.14a	8.34a
	W_3	6.32b	6.67a	5.67b	5.67c
N_1	W_1	11.06a	8.00b	7.37b	9.05b
	W_2	12.28a	9.67a	8.34a	10.42a
	W_3	9.02b	9.00a	6.37c	8.86b
N_2	W_1	9.25ab	7.00a	7.53b	7.84b
	W_2	10.32a	7.33a	8.86a	9.63a
	W_3	7.43b	7.00a	6.32c	6.32c

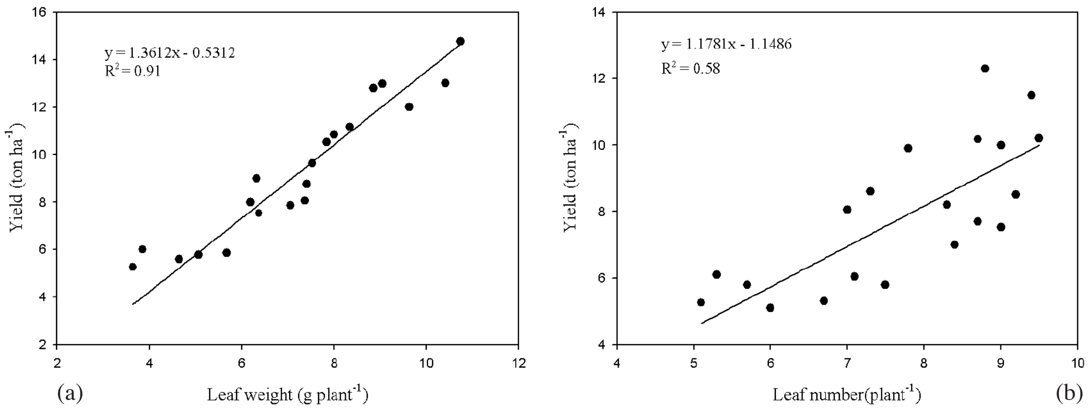

FIGURE 3. Correlation between spinach yield and leaf weight of each plant (a) and yield and leaf number of each plant (b) at different water and nitrogen treatment

TABLE 4. Water use efficiency (WUE) and nitrogen use efficiency (NUE) of spinach in different water and nitrogen treatments

Nitrogen application	Water level	WUE (kg ha^{-1} mm^{-1})	NUE (kg yield kg^{-1}N)
N_1	W_1	7.87b	62.47a
	W_2	10.10b	41.41b
	W_3	17.06a	31.76b
N_2	W_1	14.91b	14.71a
	W_2	28.14a	9.18b
	W_3	36.87a	6.53c

conditions. WUE got the highest value 36.87 kg ha^{-1} mm^{-1} in N_2 treatment and WUE increased in lower water level. But adding nitrogen amount brought about increasing nitrogen of spinach and reduction of NUE in most cases as comparing with the N_0 condition.

Conclusion

This research pointed out that water level and nitrogen fertilizer amount had evident effects on growth characteristics, spinach yield and water and nitrogen utilization. In general, water and nitrogen significantly affected the total aboveground biomass and yield of spinach. Increasing nitrogen fertilizer amount brought about raising leaf chlorophyll content and chlorophyll content obtained the maximum value in N_2 treatment. But chlorophyll content was not affected by water deficit. Spinach yield was higher at N_1 compared with N_0 and N_2 at all water levels. Abundant water supply resulted in the highest spinach yield. Leaf weight was a main reason for yield increase in all treatments. Nitrogen fertilization decreased NUE in all the treatments. WUE of spinach increased with adding nitrogen application in most conditions. WUE and NUE should be improved so that farmers got more earnings and reduced the harm to environment. However, these results indicated that sustainable production of spinach could be obtained through a proper management of water and nitrogen applications.

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