Journal of Fertilizers & Pesticides

Maneejantra et al., J Fertil Pestic 2016, 7:2
DOI: 10.4172/2471-2728.1000170

Case Report Open Access

A Quantitative Analysis of Nutrient Requirements for Hydroponic Spinach (*Spinacia oleracea L.*) Production Under Artificial Light in a Plant Factory

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Abstract

Surplus absorption of elements that contribute little to crop productivity and quality can be avoided, and fertilizer consumption costs minimized, by applying elements quantitatively to the nutrient solution fed to the plants. The aim of this study was to determine the minimum macronutrient requirements of spinach (*Spinacia oleracea* L.) with a desired plant size, so that fertilizer management in plant factories can be maximized. Spinach plants were grown in a plant factory (20°C/17°C day/night temperature, photosynthetic photon flux (PPF) of 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 12 hours per day using cool-white fluorescent lamps, 1,000 $\mu mol \cdot mol^{-1}$ CO_2). Spinach grew and developed rapidly, and reached its desired marketable size in only 12–15 days after being transplanted to the study conditions. At day 15 of cultivation under the treatment conditions, the required quantities of macronutrients per plant (90 grams in fresh weight) were determined as follows: 191 mg N, 31 mg P, 345 mg K, 34 mg Ca, 38 mg Mg, and 13 mg S. In conclusion, a quantitative nutrient managing method with low nutrient concentrations is feasible and resource-saving for hydroponic vegetable production in plant factories.

Keywords: Deep flow technique (DFT); Macronutrients; Quantitative fertilizer management

Introduction

Hydroponic systems have currently been widely established, and they are applied for vegetable and flower production worldwide. Management of the used nutrient solutions is considered as an effective way to control the quality and productivity of the crops, since nutrients are one of the main factors influencing plant growth and development. Nutrient solutions are basically composed of potassium (K), nitrogen (N), phosphorus (P), calcium (Ca), magnesium (Mg), sulphur (S), and micronutrients. In commercial vegetable production, addition of nutrients is usually controlled by the electrical conductivity (EC) of the nutrient solution. In addition, nutrients can be optimized to get a desirable level of plant growth and development. Many studies have been conducted to evaluate the effects different nutrient solutions can have on the growth, morphology, and antioxidant contents in various cultivars of tomato [1,2], and leaf lettuce [3]. Moreover, nutrient solution composition has also been modified to obtain low-potassium lettuce and tomato for dialysis patients [4,5].

Some nutrients (e.g., N, P, and K) tend to be absorbed rapidly by plants under the EC-controlled nutrient solution. However, when the absorption exceeds a threshold (luxury absorption), it will not contribute extra to the productivity and quality of the crop [3,6]. Therefore, a new concept of nutrient management for growing vegetables was proposed, in order to improve fertilizer use efficiency. In this concept, the application of fertilizer is regulated quantitatively (quantitative management). This means that specific weights of fertilizer are added to the solution tank at regular intervals, regardless of the nutrient concentrations or the solution's EC value [7,8]. Although the nutrient concentrations in such a solution are lower and fluctuate more than under the normal control method, most ions can still be absorbed rapidly by the plants, independent of the ion concentrations [9]. Therefore, plant growth will be not suppressed by this kind of fertilizer supply, whereas surplus addition can be avoided, minimizing the fertilizer costs. Thus, to apply this method to practical cultivation, the nutrient requirements of the target crop at a specific growth stage must be determined first. In this study, spinach (*Spinacia oleracea* L.), which is popular in the market, and rich in carotene and vitamin C [10], was selected as target crop. Therefore, the nutrient requirements of hydroponic spinach at a specific growth stage was determined. This knowledge provides new insights into the fertilizer application method in crop cultivation in a plant factory.

Materials and Methods

Cultivating conditions

Spinach seeds (*Spinacia oleracea* L.) were sown in a germination tray filled with granulated rock wool for substrate on July 25, 2014. The trays were placed in a growth chamber just after sowing, at 20°C/17°C (day/night). Photosynthetic photon flux (PPF) in the chamber was provided by cool-white fluorescent lamps at 350 $\mu mol\ m^{-2}\ s^{-1}$ for 12 hours per day, and CO_2 concentration was maintained at 1,000 $\mu mol\ mol^{-1}$. Seedlings were sub-irrigated twice a day with a commercial nutrient solution. After 14 days, 50 uniform plants were selected and transplanted into a hydroponic system, with 15 cm distances between the plants. In a growth room with controlled environmental conditions, a DFT (deep flow technique) system that circulated a nutrient solution enriched with O_2 by an air pump was set up for spinach cultivation. Rain water was used to prepare the nutrient solution. An 'Enshi' (horticultural experimental station) formula solution was applied to the system, and the solution's EC was adjusted to 2.2–2.4 $dS\ m^{-1}$. The concentrations of the standard 'Enshi' formula composition were 4.0 mM of $Ca(NO_3)_2.4H_2O$, 8 mM of KNO_3, 1.33 mM of $NH_4H_2PO_4$, 2.0

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Received October 17, 2016; **Accepted** October 14, 2016; **Published** October 30, 2016

Citation: Maneejantra N, Tsukagoshi S, Lu N, Supaibulwatana K, Takagaki M, et al. (2016) A Quantitative Analysis of Nutrient Requirements for Hydroponic Spinach (*Spinacia oleracea L.*) Production Under Artificial Light in a Plant Factory. J Fertil Pestic 7: 170. doi:10.4172/2471-2728.1000170

mM of $MgSO_4.7H_2O$, 205 mM of Fe-EDTA, 138 mM of H_3BO_3, 2.8 mM of $MnSO_4.4H_2O$, 3.5 mM of $ZnSO_4.7H_2O$, 0.92 mM of $CuSO_4.5H_2O$, 0.29 mM of $Na_2MoO_4.2H_2O$, and the EC was 2.4 dS m^{-1}. Growth room temperature was kept at 25°C, and the light period was 16 h. The solution temperature was around 25°C, but was not maintained. Light-emitting diode (LED) lamps were used as the light source, and the PPF just above the planting panel was set at 250 µmol m^{-2} s^{-1}. The CO_2 concentration was 1,000 µmol mol^{-1}.

Plant analysis

Plants were harvested every 3 days (until 18 days after transplantation) to measure growth parameters and conduct mineral uptake analyses. The growth parameters include chlorophyll content of the inner and outer leaves, shoot fresh weight and dry weight, root fresh weight and dry weight, total leaf number, and leaf length [11]. For leaf length, the longest leaf from each plant was measured. For the biochemical analyses, functional leaves were randomly selected. The samples' dry weights were measured after oven-drying them at 80°C for 5 days. Dried samples were subsequently ground to powder for mineral analyses. For each sample, 500 mg powder was mixed with 8 mL of concentrated nitric acid. This mixture was homogenized by using a high-performance homogenizer, and subsequently diluted with distilled water to 100 mL. Ca, K, Mg, and P contents were measured with an inductively coupled plasma analysis. N and S contents were determined with a carbon-nitrogen-sulfur combustion analysis, using sulfadiazine as the standard compound.

Results and Discussion

Spinach growth and morphology under the hydroponic system. The inner and outer leaf chlorophyll content differed significantly during the cultivating period (Table 1). At 15 and 18 days after transplanting (DAT), average leaf numbers were 26 and 37, respectively, whereas average leaf length was 17.5 and 20.0 cm, respectively.

The shoot fresh weight and dry weight rapidly increased during the period of 6-18 DAT (Figures 1 and 2), and shoot fresh weight reached its desired marketable size (20-50 g) at 12-15 DAT. The increasing trends of root fresh and dry weight were similar to those of the shoots. In the previous study, shoot fresh weight (31.2 g) and dry weight (3.22 g) were obtained in hydroponics as long as 8 weeks at 28°C water temperature [12]. This may be due to differences in the used nutrient solutions, environmental conditions, and hydroponic systems. Nonetheless, our results indicate that the cultivating method in this study is very effective to produce spinach in a short period in a plant factory.

Spinach mineral concentrations during the cultivation period. Spinach shoot N, P, K, and Mg concentrations increased as the plant shoot grew (Table 2). However, Ca and S concentrations showed relatively smaller changes.

Plants utilize N as a component for proteins, nucleic acids, nucleotides, and coenzymes, among others [13]. In addition, K is essential for enzyme activity as a cofactor, and additionally plays a role in cellular electroneutrality, photosynthesis, water and solute transportation, and osmoregulation [13,14]. Plants therefore generally require more N and K than other macronutrients [14]. Our results confirmed this, since N and K concentrations in the spinach shoot were 44.5 mg g^{-1} DW and 76.0 mg g^{-1} DW at 3 DAT, respectively, and increased to 61.6 mg g^{-1} DW and 94.3 mg g^{-1} DW at 18 DAT.

Spinach root N concentrations showed a relatively constant value throughout the cultivation period (Table 3). Root K concentration increased from 3 to 9 DAT, but gradually decreased from 9 to 18 DAT. The latter indicates that spinach required high amounts of K during the early phase after transplanting for electroneutralization and growth maintenance.

The concentrations of P, Ca, Mg, and S showed trends similar to the changes in N and K, although their concentrations were lower in both the shoots and roots (Tables 2 and 3). Spinach shoots required P in moderate amounts, and P concentrations slightly increased from 6.4 to 8.1 mg g^{-1} DW, whereas roots required 3.4 to 10.0 mg g^{-1} DW during the cultivation period (Tables 2 and 3). Shoot Ca concentrations ranged from 9.6 to 10.9 mg g^{-1} DW, although there was a fluctuation of Ca concentrations in the roots. The latter varied from 7.4 to 11.8 mg g^{-1} DW before 12 DAT, increased to 13.1 mg g^{-1} DW at 15 DAT, and dropped again to 10.7 mg g^{-1} DW at 18 DAT. Shoot Mg concentrations did not change much at 3 and 6 DAT, ranging from 7.3 to 8.0 mg g^{-1} DW, respectively. However, it slightly increased from 8.0 to 13.6 mg g^{-1} DW during 6 to 18 DAT. Although the plant's S requirements have not become clear, S concentrations ranged from 3.6 to 4.4 mg g^{-1} DW in the shoots, and varied between 4.2 and 3.8 mg g^{-1} DW in the roots.

Under the conditions of the present study, the adequate cultivation period for the spinach was considered to be 12 to 15 days. The quantities of macronutrients required to achieve the appropriate size is estimated by using shoot and root nutrient concentrations, and weights per plant (Table 4). These values can be used to design the fertilizer

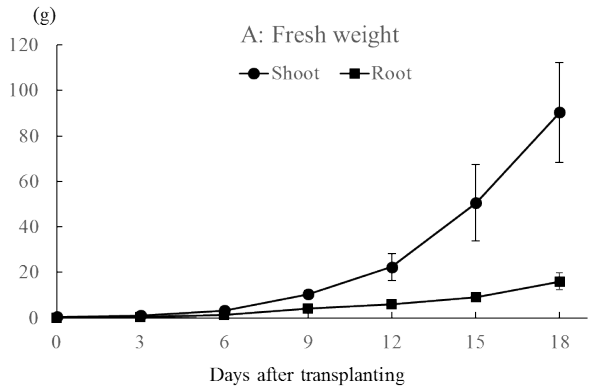

Figure 1: Spinach shoot and root fresh weight during the days after transplanting. Vertical bars indicate SD (n=3). New nutrient management for spinach.

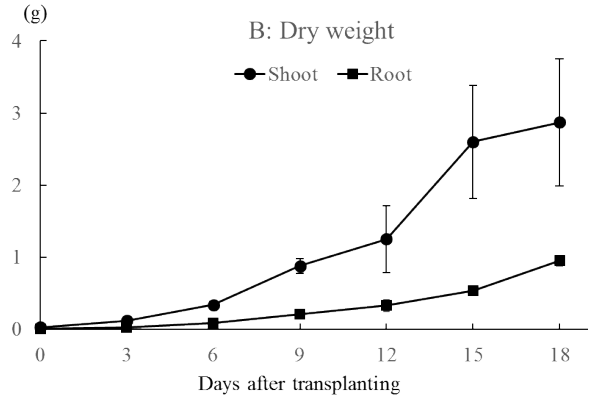

Figure 2: Spinach shoot and root dry weight during the days after transplanting. Vertical bars indicate SD (n=3). New nutrient management for spinach.

application for quantitative management in spinach cultivation in a plant factory. For example, fertilizers could be supplied to the spinach in the same proportions as the macronutrient requirements from Table 5. Furthermore, based on these values, it would be possible to control specific mineral concentrations in the products, by managing the fertilizer quantity.

Conclusions

In general, studies on mineral requirements during each growth period are essential for agricultural improvements. For cultivating leafy vegetables in hydroponics in a plant factory, the total quantity of mineral requirements during the growth period is more important, since the cultivation period is very short. Even though there are various nutrient solution formulas, plants generally require different amounts of macronutrients. This study determined the required macronutrient quantities for spinach in a DFT hydroponic system. The mineral uptake of spinach increased with plant growth throughout the cultivation period. K and N requirements were higher than the requirements of other elements. Furthermore, the standard value for designing fertilizer applications for plant factory spinach production was determined. This knowledge will provide a better understanding of developing new

DAT[1]	Chlorophyll content (SPAD units)		Leaf No.	Leaf length (cm)
	Outer leaves	Inner leaves		
3	48.5 ± 2.7[2]	48.2 ± 4.2	4 ± 0	7.0 ± 0.6
6	53.1 ± 4.2	51.2 ± 5.2	7 ± 1	8.9 ± 1.0
9	51.3 ± 2.2	50.6 ± 3.9	11 ± 1	10.3 ± 0.7
12	51.0 ± 3.3	48.3 ± 2.3	14 ± 1	14.0 ± 1.1
15	48.3 ± 3.0	46.5 ± 2.5	26 ± 5	17.5 ± 3.0
18	46.5 ± 2.4	47.5 ± 0.8	37 ± 7	20.0 ± 1.2

Table 1: Morphological development of spinach leaves after transplantation to the hydroponic system.

DAT[1]	N (mg.g^{-1} DW)	P (mg.g^{-1} DW)	K (mg g^{-1} DW)	Ca (mg g^{-1} DW)	Mg (mg g^{-1} DW)	S (mg g^{-1} DW)
3	44.5 ± 2.0[2]	6.4 ± 1.2	76.0 ± 8.8	9.8 ± 0.9	7.3 ± 0.8	3.6 ± 0.6
6	51.4 ± 4.3	7.2 ± 1.5	94.3 ± 11.8	9.4 ± 0.3	8.0 ± 1.7	3.6 ± 0.0
9	59.7 ± 1.9	8.8 ± 0.3	118.7 ± 5.5	9.2 ± 1.0	10.8 ± 1.0	3.8 ± 0.1
12	63.2 ± 1.3	8.6 ± 0.7	103.7 ± 8.8	8.2 ± 1.1	11.2 ± 1.2	4.0 ± 0.2
15	62.3 ± 0.8	9.4 ± 0.2	117.0 ± 2.8	8.3 ± 1.1	12.8 ± 1.0	4.1 ± 0.3
18	61.6 ± 3.8	8.1 ± 1.2	94.3 ± 16.2	10.9 ± 2.8	13.6 ± 2.7	4.4 ± 0.4

[1]Days after transplanting.
[2]Mean ± SD (n=3)

Table 2: The concentration of macronutrients in spinach shoots throughout the cultivation period.

DAT[1]	N (mg g^{-1} DW)	P (mg g^{-1} DW)	K (mg g^{-1} DW)	Ca (mg g^{-1} DW)	Mg (mg g^{-1} DW)	S (mg g^{-1} DW)
3	56.2[2]	3.4	79.3	7.5	2.4	4.2
6	63.2	10.3	73.1	11.8	5.1	3.7
9	58.9 ± 0.6[3]	11.5 ± 1.9	90.2 ± 7.8	10.7 ± 2.0	6.4 ± 0.8	3.5 ± 0.3
12	57.6 ± 1.8	9.9 ± 0.3	77.9 ± 2.7	7.9 ± 0.4	6.3 ± 0.3	3.4 ± 0.2
15	53.8 ± 5.1	11.6 ± 0.6	75.2 ± 10.3	13.1 ± 2.4	8.8 ± 1.1	3.5 ± 0.3
18	52.5 ± 1.6	10.0 ± 0.9	70.7 ± 5.8	10.7 ±1.0	7.4 ± 0.5	3.8 ± 0.3

[1]Days after transplanting.
[2]Not statistically analyzed because of insufficient replicates.
[3]Mean ± SD (n=3)

Table 3: The concentration of macronutrients in spinach roots throughout the cultivation period.

DAT[1]	N	P	K	Ca	Mg	S
(mg plant^{-1})						
12	98.0	14.0	155.3	12.9	16.1	6.1
15	191.0	30.7	344.8	34.1	38.0	12.6
(mmol plant^{-1})						
12	7.0	0.5	4.0	0.3	0.7	0.2
15	13.6	1.0	8.8	0.8	1.6	0.4

Table 4: Estimated macronutrient requirements of spinach to achieve the marketable size in a hydroponics system.

DAT[1]	$Ca(NO_3)_2$ $4H_2O$	KNO_3	$NH_4H_2PO_4$	$Mg(NO_3)_2$ $6H_2O$	$MgSO_4$ $7H_2O$	$NaNO_3$	$MgCl_2$ $6H_2O$
(mg plant^{-1})							
12	70.8	404.0	57.5	128.0	49.2	76.5	-
15	188.8	888.8	115.0	281.6	98.4	-	20.3

[1]Days after transplanting.

Table 5: Fertilizer combination examples according to the estimated macronutrient requirements of spinach.

fertilization methods, as well as new approaches for improving future plant factory crop growth.

Acknowledgments

This work was supported by the Kieikai Research Foundation to Dr. Wataru Yamori.

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