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Full Length Research Paper

Effects of nitrogen and light intensity on tomato (*Lycopersicon esculentum* Mill) production under soil water control

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Light, water and nitrogen supply are important factors affecting crops production and growth. To evaluate the effect of different light intensities and nitrogen supply levels with different soil water content on the production of tomatoes, a pot experiment was conducted on the Zijingang campus of Zhejiang University in Hangzhou, China, during the tomato-growing season in 2009. Results showed that the higher the light intensity, the higher the production of fruits under the same nitrogen treatment and the best conditions for optimal yield were: 60% of the traditional nitrogen application, high light intensity and 70-75% soil water content. Under 70 - 75% soil water, nitrate reductase (NR) decreased along with the increased NO_3-N supply level, and the contents of soluble sugar and ascorbic acid increased with increased light intensity. This suggested that modulating the relationship between soil water content, light intensity and nitrogen supply level could increase the production and promote fruit quality.

Key words: Tomato, light intensity, nitrogen supply, soil water content, biomass.

INTRODUCTION

Light intensity, soil water content and nitrogen supply are important factors affecting the biomass of crops, and also have a direct impact on photosynthesis and growth. However, how to maximize their efficiency to enhance crop growth has been a crucial topic in the field. The composition of the fruit is markedly affected by light levels, which also affects the fruits ripening characteristics including harvest time and storage life (Tombesi et al., 1993; Zoran et al., 2012). Diversified light intensity could have different effects on the development of leaf area, growth and yield (Vyas et al., 1996; Martin et al., 2011). Popp (1926) found that in *heliophilous* plants, the rate of material accumulation increased with an increase in light intensity, while any increase beyond optimal light intensity resulted in a decrease in the rate. Also, if grown under weak light intensity, the kiwi fruits need more time to reach the commercial harvest stage with paler color, smaller size and poorer sensory characteristics than in full light (Antognozzi et al., 1995). The plants grown in high light intensity resulted in high quality fruits which could be stored for a long time (Antognozzi et al., 1995). At the same time, insufficient shade can affect productivity by causing more light to be incident on the plant, bringing about photo inhibitory effects that harm the metabolic process of the system (Vyas, 2004). Therefore, it is necessary to investigate the optimal light conditions to maximize productivity (Puthur, 2005).

Drought conditions may restrain nitrate acquisition by the roots, as well as the ability of the plants to assimilate nitrogen (Frechilla et al., 2000). Tomato plants grown under conventional deficit irrigation exhibited water stress and led to significant reduction in tomato yield which resulted in gas exchange rate reduction (May and Gonzalerz, 1999). In partial root drying (PRD) practice, the activity of nitrate reductase (NR) increased in wheat under polyethylene glycol (PEG) mild stress, while the

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activity decreased under severe stress. This reaction could be managed to adjust the production of amino acids and sugars according to the demand under stress conditions in combination with limited nitrogen supply (Kocheva et al., 2007). Nitrate is the predominant form of nitrogen available to plants; nitrogen is an important component of chlorophyll, protein, nucleic acid, and some hormones in plant, and it also controls the growth and development of plant in many ecosystems. The productivity of plants is largely influenced by the assimilation of NO_3^- nitrogen (Yasuo, 1996; Yin et al., 2011). Increasing the amount of nitrogen supply raises the rate of photosynthesis and the light saturation point, thus increasing chlorophyll content and biomass (Yin et al., 2011). According to Zotarelli et al. (2009a, b), 87% of the tomato yield did not increase above 224 kg/ha of N, and use of N application rates above 220 kg/ha did not result in fruit and/or shoot biomass nor N accumulation benefits. A research by DaMatta et al. (2002) indicates that low N treatment causes an increase in cell wall rigidity and osmotic adjustment. Both mechanisms might improve the extraction of water from drying soil, in addition to avoiding excessive loss of cell volume, thus leading to some degree of drought tolerance (Damatta et al., 2002). However, an excessive nitrogen supply will in some species lead to luxury consumption and nitrate accumulation in cells, and the plant yield cannot be increased between 300 kg/ha nitrate application to 600 kg/ha nitrate application (Maynard et al., 1976; Lou et al., 2012).

Existing research has only proved that the best approach to increasing yield is the optimal integration of the three factors. Research focused on the interaction of the three factors is limited. Green leafy vegetables are always fertilized with abundant nitrogen to gain high yields, especially in China. The traditional applied N quantity is 600 - 900 kg/ha (Lou et al., 2012). However, an excessive nitrogen supply will lead to luxury consumption and nitrate leaching into ground water, which is known to be a problem in vegetable production (Lou et al., 2012). The orthogonal matrix method to determine the optimal setting has been applied to study the relations between the experimental variables and their effects on biomass accumulation (Escamilla et al., 2000; Xu et al., 2003). Orthogonal design is a type of experimental design method which uses the Taguchi parameter design methodology (Montagomery, 1991, 1996). It is easy to investigate the influence of controlled factors in a multivariable system using this method. In addition, the orthogonal matrix can simultaneously investigate many more factors than for instance central composite design, and thus facilitates economical benefit and experimental convenience, while being competitively rapid and precise (Joo et al., 2004). The notation L_a (b^c) is used to represent the orthogonal array, where 'a' is the number of experimental runs, 'b' the number of levels for each factor or variable and 'c' is the number of factors investigated (Escamilla et al., 2000).

In this study, the effects of nitrogen fertilizer application, soil water content and light intensity on the biomass accumulation of tomatoes were evaluated by orthogonal design. The aim is to reduce the traditional nitrogen fertilizer application without reducing production through the appropriate combination of water and light intensity, and to provide a basis for the study of the relationship between these three factors, and establish a protocol for production practice.

MATERIALS AND METHODS

The experiment was conducted in tomato-growing season 2009 (from early March to early August) at the Zijingang campus experiment station of Zhejiang University in Hangzhou, China (30°46´-30°50´N, 119°46´-119°48´E). Soil samples were taken from a fallow field in the North China, with soil pH of 7.60, alkali-hydrolysable nitrogen 59.33 mg/kg, active phosphorus 43.28 mg/kg and active potassium 166.77 mg/kg. Tomato seeds (*Lycopersicon esculentum* Mill) were soaked in water for 24 h and germinated on moistened filter paper in Petri dishes at 25°C for another 48 h. Germinated seeds were planted in growth holes mixed with medium vermiculite and medium turve (1:1, v/v) proportionally. Each plant was watered in the morning with distilled H_2O under controlled conditions with a 12 h light period, a 23/18°C light/dark temperature regime and 50% relative humidity for 40 days. The forty-day-old seedlings were transplanted on 28 April 2009, into 25 × 25 cm round single plastic pots holding 10 kg of soil.

Imposition of stress

Urea, phosphate fertilizer and potash fertilizer were applied as the basal fertilizers, which were all added with 600 kg/ha to the soil, and the following factors were studied: (a) three levels of nitrogen fertilization: 100 kg/ha equal to 0.057 gN/kg soil (N1), 350 kg/ha equal to 0.142 gN/kg soil (N2) and 600 kg/ha equal to 0.342 gN/kg soil (N3) in the form of urea; (b) three levels of relative water content in the soil: 55 - 60% (H1), 70 - 75% (H2) and 85 - 90% (H3). Relative soil water content defined as the amount of water available relative to the amount of water at field capacity was employed here as a general measure of soil water availability (Reichstein et al., 2003). (c) Three levels of light intensity to simulate three kind of weather conditions: 1150 μmol/m^2·s (L3), 378 μmol/m^2·s (L2) and 115 μmol/m^2·s (L1). Different light intensities were shaded with two different shading nets, which were used to make the 65 and 90% of sunlight, respectively (Zoran et al., 2012).

Orthogonal design method

The experiments were performed at three nitrogen supply levels (A-kg/ha), three light intensity levels (B-μmol/m^2·s) and three soil water content levels (C-%): 1. A-100(N1), B-115(L1), C-55-60% (H1); 2. A-350(N2), B-368(L2), C-70-75% (H2); 3. A-600(N3), B-1150(L3), C-85-90% (H3). The experiments were carried out by L_9 (3^4) orthogonal array (Table 1).

Plant sampling

The tomatoes were harvested at physiological maturity. Commercial yield was determined by weighing only marketable fruits on each plant. The term 'marketable' or 'commercial' indicated fruits with acceptable color, caliber and firmness (Ruiz and Romero, 1998).

Table 1. Arrangements of the L_9 (3^4) Latin square.

Experiment no.	Factor			Tomato yield (kg/ha)
	A	B	C	
1	N1	L3	H3	43536 ± 3779
2	N1	L2	H2	16266 ± 1100
3	N1	L1	H1	0
4	N2	L2	H3	20505 ± 1940
5	N2	L1	H2	3909 ± 468
6	N2	L3	H1	570778 ± 4135
7	N3	L1	H3	0
8	N3	L3	H2	55070 ± 1750
9	N3	L2	H1	17174 ± 798

The nitrate content in the tomato was analyzed according to Lang (1958) in 100 mg of tissue oven-dried at 70°C for 72 h. Plant material was digested with tetraoxosulphate (VI) acid (H_2SO_4) and subsequently oxidized with hydrogen peroxide (H_2O_2). Aliquots from the extract were reacted with potassium hydroxide (KOH) and Nessler's reagent. The resulting absorbance was read at 440 nm.

Soluble sugars (sucrose, glucose, and fructose) and ascorbic acid content of the fruit were determined as described by Saglio (1980) and Law (1983), respectively. Soluble sugars (sucrose, glucose and fructose) were extracted by boiling ethanol (80% [v/v] in H_2O_2), and determined successively by a specific enzymic method. The ascorbic acid assay was conducted using the 2,6-dichlorophenol-lindophenol (DCIP) photometric method. Briefly, 1 g fresh sample was ground in 10 ml of ice-cold 0.0005 M disodium ethylenediamine tetraacetic acid (EDTA) solution containing 3% trichloroacetic acid (TCA) for 1 - 2 min. The homogenate was quickly filtered and brought up to 20 ml with EDTA-TCA extracting solution. Subsequently, 1 L of distilled water, 2 ml of 4-(3,5-dichloro-4-hydroxyphenyl)iminocyclohexa-2,5-dien-1-one (DCIP) reagent and 2 ml of filtered extract were added to each test tube and their optical densities at 600 nm were determined from a standard curve previously prepared using various determined concentrations of the ascorbic acid.

Titratable acidity was determined by titrating with 0.1 N sodium hydroxide (NaOH) (Mitchell and Shennan, 1991). Nitrate reductase (NR) (EC 1.7.1.1) was measured by the *in vitro* method described by Leleu et al. (2000), which was assayed with 0.2 g frozen samples ground in a mortar and homogenized with 2 ml 50 mM HEPES KOH (pH 7.5) buffer containing 0.5 mM EDTA, 5.5 mM magnesium chloride ($MgCl_2$), 14 mM β-mercaptoethanol, 0.1% Triton X100 (v/v), 10% glycerol (v/v), 10% polyvinylpyrrolidone (w/v), 50 µM leupeptin and 0.5 mM phenylmethanesulfonyl fluoride (PMSF). After centrifugation at 5 000 × g for 20 min at 4°C, the supernatant was assayed for NR and the NR activity was expressed as sodium nitrite ($NaNO_2$) µg/h·g.

Photosynthetic indexes measurements

A LI-6400 portable photosynthesis system (LiCor-6400: LiCor Inc. Lincoln, Nebraska, USA) was used to measure net photosynthetic rate (Pn) on the abaxial surface of leaf 4 on 4 plants per treatment, providing a photon flux density of 800 ± 1 µmol/m^2·s in the leaf chamber. Air temperature was 25 ± 1°C during the day.

Statistical analysis

Statistical analyses were performed using the SPSS13.0 program. The Tukey's test for analysis of variance (ANOVA) was considered significant at the 0.05 probability level. The output data was presented as mean values ± Error standard (SE).

RESULTS

Effects of different nitrogen fertilizer application, light intensity and soil water content levels on tomato production

The vegetative weight (kg/ha fresh weight) of the tomatoes treated with 100% natural lighting intensity (L3) was significantly higher than that of the tomatoes treated with other light intensity levels (L1, L2) under low soil water conditions (Figure 1A). Weak light intensity (L1) yielded no harvest under the condition at 100 kg/ha nitrogen supply, 55~60% soil water content (N1H1) and 600 kg/ha nitrogen supply, 85%~9% soil water content (N3H3). These results indicate that the growth and development of tomatoes is highly dependent on high light intensity (Figure 1A). No significant difference in tomato production was detected when the nitrogen supply was reduced from 600 kg/ha nitrogen (N3) to 350 kg/ha (N2) under 100% natural light intensity (L3), but there were yield differences between the two soil water content conditions. On the contrary, the tomato production for the nitrogen level of 350 kg/ha (N2) was higher than the 600 kg/ha nitrogen level (N3).

Experimental results of L_9 (3^4) orthogonal matrix method

In our experiment, three factors are the nitrogen supply, light intensity, soil water content. The three levels of the factors associated with the orthogonal matrix are shown in Table 1. The effect of those environment factors on tomato growth was calculated using the data in Table 2, which is shown in Figure 1B. The order of effect of all factors on tomato growth could be determined according to the magnitude order of R. The importance of effects of

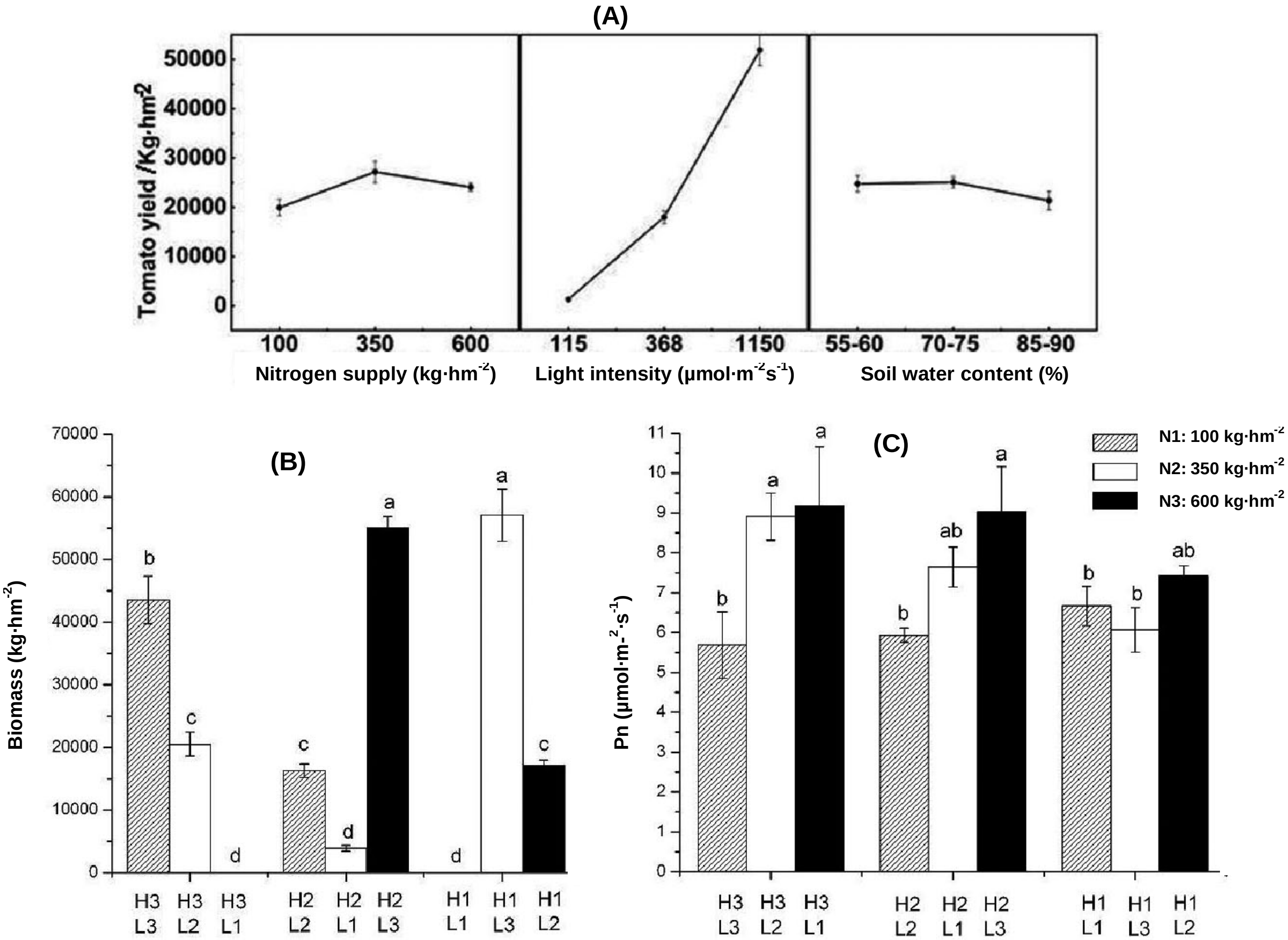

Figure 1. (A) Effects of different nitrogen, light intensity and soil water content level on the fresh fruit biomass in soil culture. (B) Intuitive analysis of the relationship between tomato yield and environment factors by pot experiment. (C) Effects of different nitrogen, light intensity and soil water content level on the net photosynthetic rate (Pn in μmol/m^2·s) of tomato plants in soil culture. Means and standard error of the means (n=4) are presented. Differences were determined using Tukey's test. Means with different letters are significantly different ($p<0.05$). (L1: 115 μmol/m^2·s; L2: 378 μmol/m^2·s; L3: 1150 μmol/m^2·s; H1: 55~60% soil water content; H2: 70~75% soil water content; H3: 85~90% soil water content).

Table 2. Analysis of experiments according to the orthogonal matrix methods.

Data	Tomato yield (kg/ha)		
	A	B	C
$K1^a$	59803 ± 4880	3909 ± 468	74251 ± 4932
$K2$	81492 ± 6543	53946 ± 3838	75246 ± 3318
$K3$	72244 ± 2547	155684 ± 9663	64042 ± 5719
$k\,1^b$	19934 ± 1627	1303 ± 156	24750 ± 1644
$k\,2$	27164 ± 2181	17982 ± 1279	25082 ± 1106
$k\,3$	24081 ± 849	51895 ± 3221	21347. ± 1907
R^c	7230 ± 1332	50592 ± 3065	3735 ± 801
Optimal level	N2	L3	H2

[a] ki = ∑k of all experiment at the same factor level. Values are mean of triple determinations with standard deviation (±); [b] Average of *ki*. Values are mean of triple determinations with standard deviation (±); [c] R = max{average of ki})min{average of ki}. Values are mean of triple determinations with standard deviation (±).

Table 3. Effects of nitrogen, light intensity and soil water content level on the tests of the Between-subject effects in the unvaried analyses from SPSS.

Source	Type III sum of square	*F*	Sig.
Nitrogen supply	7.9E7	3.5	0.221
Soil Water content	2.6E7	1.1	0.446
Light intensity	4.0E9	178.4	0.006

factors on tomato growth was light intensity>nitrogen supply>soil water content. We could conclude from the result that the effect of light intensity was more important than that of other environmental factors.

The tests of the between-subject effects (Table 2 and 3) from SPSS showed that light had a significant effect on the weight of the plant ($P<0.05$). The optimization levels of each factor using the data in Table 2 is as follows: 1150 μmol/m^2·s (L3) for light intensity, 70-75% (H2) for soil water content and 350 kg/ha for nitrogen supply (N2).

Fruit nitrogen content and quality analysis

The nitrate content (Figure 2A) in fruits at the same soil water content level (H2) showed a gradual increase with increased nitrogen supply. Since no product was harvested under weak light intensity conditions (L1N1H1, L1N3H3), the trend of the nitrate content under other soil water content levels (H1 and H3) cannot be compared. The fruit nitrate content is closely related to nitrogen assimilation capacity (Yin et al., 2011; Lou et al., 2012). Under the same soil water content conditions, increased nitrogen supply levels correlate to increased nitrate content. The fruit nitrate content increased as the nitrogen supply levels increased from N1 to N3 under the same light intensity levels (L2 and L3), indicating that high nitrogen supply levels promote high nitrate accumulation (Yin et al., 2011; Lou et al., 2012). Compared with low light intensity (L1), high light intensity (L3) was particularly favorable for the accumulation of soluble sugars under the same soil water content level (H2). The soluble sugar content was between L1 and L3 treatments for the mild light intensity (L2). An almost 1.6-fold increase in soluble sugars was found in plants at the 350 kg/ha nitrogen supply level (N2) and high light intensity treatment (L3), compared with weak light intensity (L1) (Figure 2B). All the results indicate that the high nitrogen supply level facilitated the accumulation of soluble sugars. Under high light intensity (L3), soluble sugars increased almost 1.2-fold at the 600 kg/ha nitrogen supply level (N3) compared with 100 kg/ha nitrogen supply level (N1).

The change in ascorbic acid (Figure 2C) showed a trend similar to that of soluble sugars. High light intensity (L3) fruits increased in ascorbic acid accumulation compared to those grown in weak and mild light intensities (L1, L2) under different soil water content and the same nitrogen supply level. Also, compared with low light intensity (L1), high light intensity (L3) was particularly favorable for ascorbic acid accumulation under the same soil water content level (H2). The effect of mild light intensity (L2) was between that of the L1 and L3 treatments. Under high light intensity (L2 and L3) conditions, ascorbic acid content increased as the nitrogen supply level increased from N1 to N3. The ascorbic acid content at the 350 kg/ha nitrogen supply level (N2) showed the optimum accumulation (Figure 2D).

Changes in enzyme activity

Plants grown in high light intensity (L3) exhibited higher NR activity than those grown in weak and mild light intensity (L1, L2) under different nitrogen supply and soil water content levels (Figure 3). At the 350 kg/ha nitrogen supply (N2) level, NR activity in high light intensity (L3) showed the maximum value in the experiment, with 1.8 times higher than in mild light intensity (L2). Under high light intensity (L2 and L3) conditions and different soil water content levels, NR activity first increased and then decreased as the nitrogen supply level increased from N1 to N3. NR activity reached optimum accumulation at the 350 kg/ha nitrogen supply level (N2), which especially favorable the production.

DISCUSSION

The vegetative weight (kg/ha fresh weight) of the tomatoes grown under 100% natural light intensity was significantly higher than that of the tomatoes treated with other light intensity levels (L1, L2) with the same nitrogen supply. High light intensity (L3) conditions promoted the fruit production. The quantity of nitrogen fertilization could be properly reduced to 60% of the traditional nitrogen supply level in the presence of appropriate soil water content (H2) and adequate light intensity supply (L3), which is significantly important to decreasing fertilizer use. Our results found that the reduction in nitrogen fertilizer application would be quite practical and economical, especially in agricultural production in modern China.

In this study, the net photosynthetic rate (Figure 1C) increased gradually when the nitrogen supply level

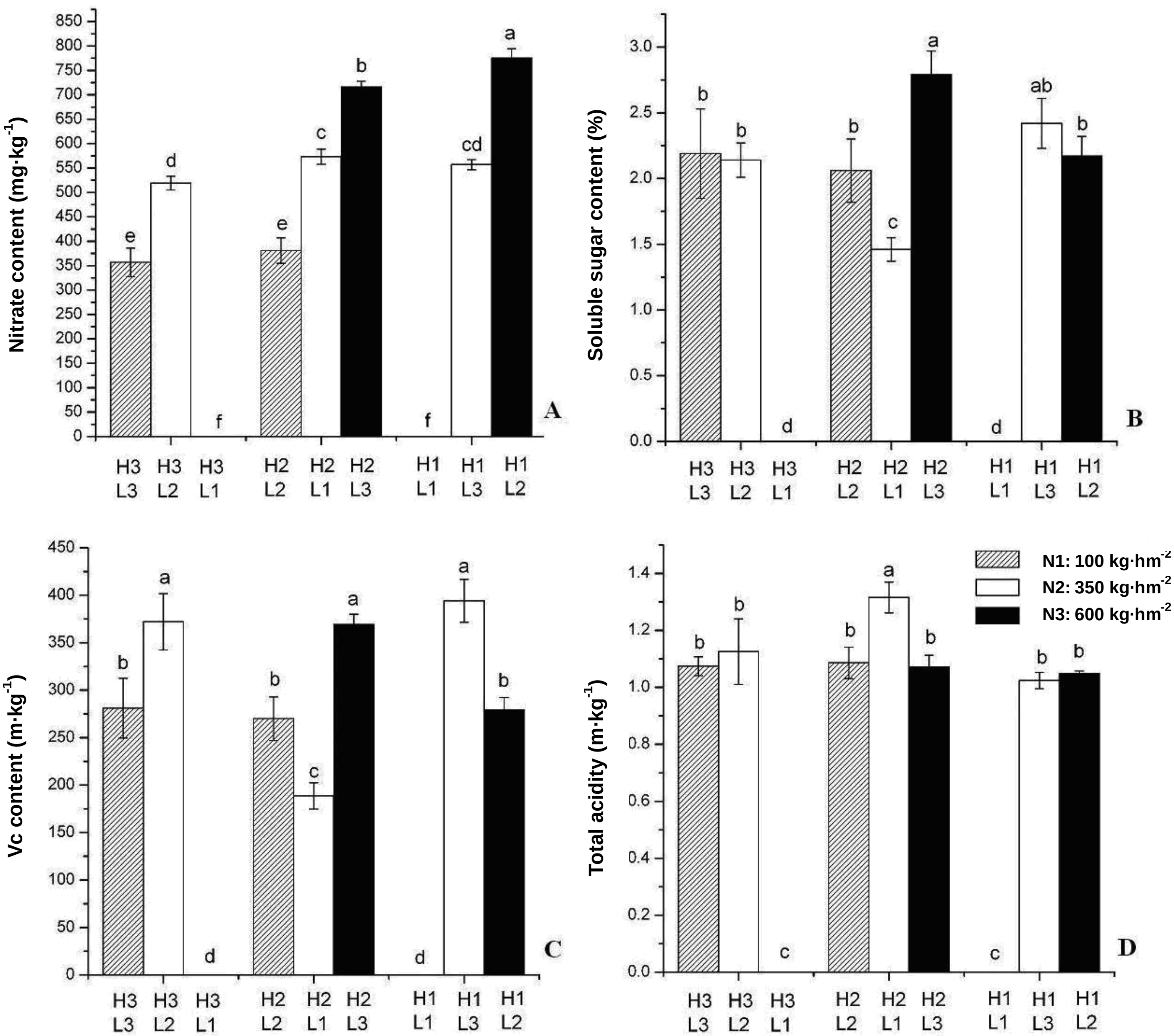

Figure 2. (A) Effects of different nitrogen, light intensity and soil water content level on the content of nitrate of tomato in soil culture. (B) Effects of different nitrogen, light intensity and soil water content level on the content of soluble sugar of tomato in soil culture. (C) Effects of different nitrogen, light intensity and soil water content level on the content of ascorbic acid (Vc) of tomato in soil culture. (D) Effects of different nitrogen, light intensity and soil water content level on the content of titratable acidity of tomato in soil culture. Means and standard error of the means (n=4) are presented. Differences were determined using Tukey's test. Means with different letters are significantly different ($p<0.05$). (L1: 115 μmol/m^2·s; L2: 378 μmol/m^2·s; L3: 1150 μmol/m^2·s; H1: 55~60% soil water content; H2: 70~75% soil water content; H3: 85~90% soil water content).

increased from N1 to N3. The 600 kg/ha which indicated the nitrogen supply (N3) promote the high photosynthetic rate. The relationship between photosynthesis rate and yield examined appeared to support the report of Zelitch (1982). Hence, it was concluded that there is a no relationship between photosynthesis and yield. Nitrate not taken up by a crop may potentially contribute to ground and surface water pollution through nitrate leaching and soil erosion (Wang et al., 2002). Our results also showed that the content of nitrate in fruits is closely related to nitrogen accumulation. The increase of nitrogen fertilizer application showed a gradual increase in plant nitrate content under the same light intensity level (Lou et al., 2012). Fruit nitrate concentration rose with increasing nitrate application supply without great improvement in production. In agreement with the

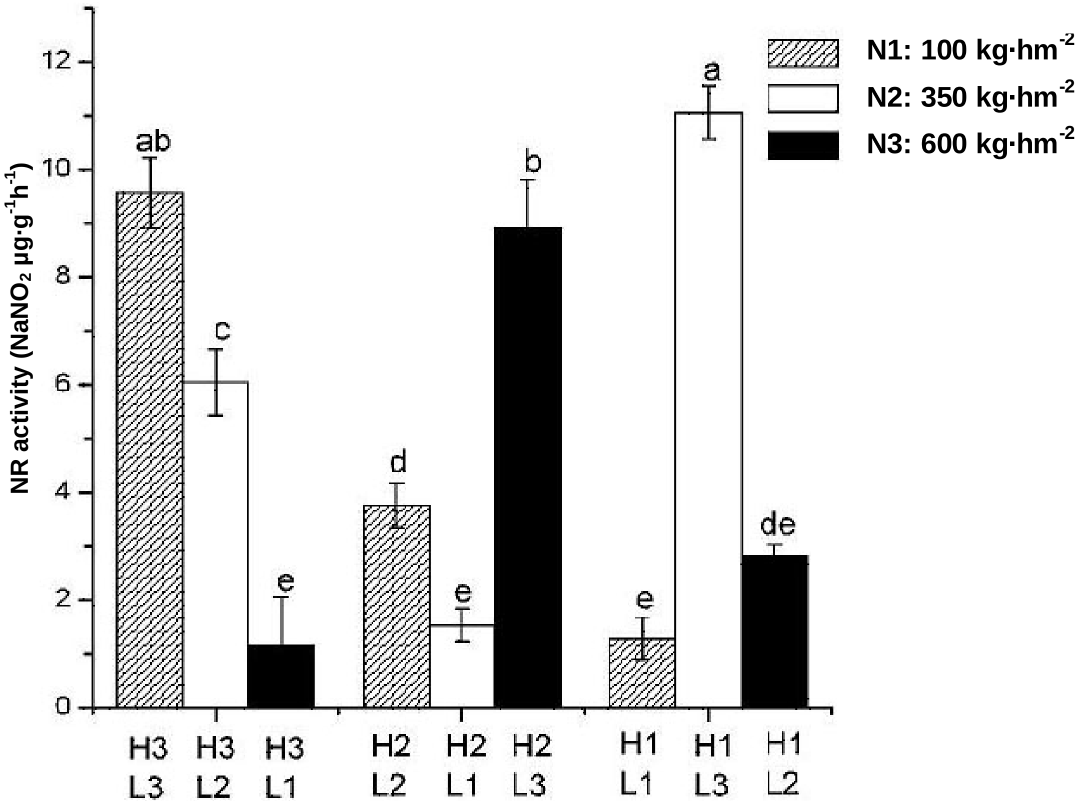

Figure 3. Effects of different nitrogen, light intensity and soil water content level on NR activity of tomato plant in soil culture. Means and standard error of the means (n=4) are presented. Differences were determined using Tukey's test. Means with different letters are significantly different ($p<0.05$). (L1: 115 μmol/m^2·s; L2: 378 μmol/m^2·s; L3: 1150 μmol/m^2·s; H1: 55~60% soil water content; H2: 70~75% soil water content; H3: 85~90% soil water content).

present results, Chen et al. (2004) found that plants grown with a high nitrate supply exhibited toxicity symptoms and depressed growth, but had higher nitrate concentrations in the whole plant dependent on exogenous nitrate, which might be mainly those of plants with maximum yield. Furthermore, many scientists have investigated the effects of nitrate reduction on higher plants and found that nitrate reduction occurred simultaneously with the fixation and reduction of carbon dioxide (Eichelmann et al., 2011; Bhupinder and Binod, 2012). Wheat leaves could reduce nitrate in the light but not in the dark (Hageman and Flesher, 1960). In our experiment, the fruit nitrate content under the N2H1L3 treatment was far lower than in the N3H1L2 treatment, which could prove the light reducing nitrate theory. There is some evidence that the NRA of corn grown under the artificial shade decreases roughly in the proportion to the amount of shading. Beside light, nitrate is also necessary for the induction of NR (Chen et al., 2004).

As the product of the carbon metabolism, soluble sugars are influenced by the combined effects of photosynthesis, carbon metabolism and nitrogen metabolism. Ascorbic acid is best known for its function as an antioxidant and for its role in collagen synthesis (Oluwafemi, 2008). Collagen deficiency results in the symptoms of scurvy (Smirnoff, 1996). Ascorbic acid is a strong determinant of fruit acidity (Akl et al., 1995). We found significant differences between the biomasses at varying light intensity levels, and soluble sugars and ascorbic acid were deeply affected by the light intensity. High light intensity promotes fruit quality and helps to increase the fruit biomass. This study, therefore, suggests that modulating light intensity, nitrogen supply and soil water content level could adjust the nitrogen assimilation capacity and the enzyme activities, which would lead to increased biomass and fruit quality.

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