ผลของไนโตรเจนที่มีต่อคุณภาพผลผลิตส้มโอพันธุ์ทับทิมสยาม

Effect of nitrogen on fruit quality of pummel (*Citrus grandis* (L.) Osbeck) cv. Tubtim Sayam

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บทคัดย่อ

ผลของไนโตรเจนที่มีต่อคุณภาพผลผลิตส้มโอพันธุ์ทับทิมสยาม ดำเนินการระหว่างเดือนมกราคม 2556 ถึง กุมภาพันธ์ 2557 ในแปลงส้มโอของเกษตรกรในตำบลคลองน้อย อำเภอปากพนัง จังหวัดนครศรีธรรมราช โดยมีวัตถุประสงค์เพื่อทดสอบการใช้ปุ๋ยไนโตรเจน 3 อัตรา คือ 1) ไม่ให้ปุ๋ย (T1) 2) การให้ปุ๋ยไนโตรเจนตามการให้ปุ๋ยของเกษตรกร(T2) และ 3) การให้ปุ๋ยไนโตรเจนตามการให้ปุ๋ยของเกษตรกรและเพิ่มแคลเซียมไนเตรทอัตรา 40 กิโลกรัมต่อไร่ (T3) ทำการเก็บส้มโอเมื่อผลอายุ 28 สัปดาห์หลังจากดอกบาน จากการศึกษาพบว่าการไม่ให้ปุ๋ยและการให้ไนโตรเจนอัตราต่าง ๆ ไม่มีผลต่อน้ำหนัก ความกว้าง ความสูง ความหนาเปลือก น้ำหนักสดเนื้อ น้ำหนักสดเปลือก น้ำหนักแห้งเนื้อ น้ำหนักแห้งเปลือก ปริมาณของแข็งที่ละลายน้ำได้ ปริมาณกรดทั้งหมด และวิตามินซีของผลส้มโอ การให้ปุ๋ยไนโตรเจนตามการให้ปุ๋ยของเกษตรกรให้ผลส้มโอที่มีน้ำตาลซูโครส กลูโคส ฟรุกโตส และน้ำตาลทั้งหมดสูงสุด 124.06, 50.61, 16.59 และ 191.26 กรัมต่อลิตรตามลำดับ แตกต่างอย่างมีนัยสำคัญทางสถิติกับส้มโอที่ไม่ได้รับปุ๋ย การให้ปุ๋ยไนโตรเจนตามการให้ปุ๋ยของเกษตรกรและเพิ่มแคลเซียมไนเตรทให้ผลส้มโอที่มีลิโมนินต่ำ 1.99 มิลลิกรัมต่อลิตร และให้ไลโคปีน (2.38 มิลลิกรัม/100 มิลลิลิตร) และแอนโทรไซยานิน (7.01 มิลลิกรัม/100 มิลลิลิตร) สูงสุด ในขณะที่ผลส้มโอที่ไม่ได้รับปุ๋ยให้เบต้า-เคโรทีนสูงสุด (0.54 มิลลิกรัม/100 มิลลิลิตร)

ABSTRACT

Effect of nitrogen on fruit quality of pummelo cv. Tubtim Sayam was carried out between January 2013 to February 2014 at a pummelo orchard in Sub-district Klong-Noy, Pak Panang District, Nakhon Si Thammarat Province. The objectives of the study were to examine the nitrogen rate that affect on fruit quality. The study in relation to application of nitrogen,

there were three treatments: no application of nitrogen-fertilizer (T1), application of nitrogen at farmers'rate (T2), and application of farmers'rate with additional 40 kg of calcium nitrate per rai (T3).Tubtim Sayam pummelo fruits were harvested 28 weeks after the flowers bloomed, it was found that those from the three treatments did not show significant difference in their weight, width, height, peel thickness, fresh pulp weight, fresh peel weight, dry pulp weight, dry peel weight, total soluble solid (TSS), total acid (TA), andvitamin C. The trees receiving nitrogen at farmers'rateproduced fruits which had high levels of sucrose, glucose, fructose, and total sugar as 124.06, 50.61, 16.59, and 191.26 g/l. respectively, significantly different from the trees receiving no fertilizer. As for the trees receiving nitrogen and additional calciumnitrate, their fruits had the lowest level of limonin (1.99 mg/l) and highest levels of lycopene and anthocyanin (2.38 and 7.01 mg/100 ml). The fruits of the trees receiving no fertilizer had the highest level of β-Cerotene (0.54 mg/100 ml).

คำสำคัญ: ไนโตรเจน คุณภาพผล ส้มโอ ทับทิมสยาม

Keywords: Nitrogen, Fruit quality, Pummelo, Tubtim Sayam

INTRODUCTION

Tubtim Sayam pummelo, which originated in Yarang District, Pattani Province, has been spreaded to Pak Panang District, Nakhon Si Thammarat Province. Its outstanding characteristics include big dark green leaves with many soft hairs on both sides, round fruits with a small knot on the top of each, yellowish green smooth skin with dense tiny oil glands, light pink thin peel, tight rows of small juicy pulps in colors ranging from dark pink to cooked-shrimp red, and sour-sweet taste (Kaewtubtim and Issarakraisila, 2011; Maneepong, 2009; Promotion and Development, 2009). Tubtim Sayam pummelo was originally grown in a small orchard with other cultivars such as Khaw Thong Dee, Roti, and Baan Yha as well as other kinds of fruit trees like mangosteens, durians, longkong and rambutans. The quality of Tubtim Sayam fruits grown in Pattani areas is generally low, possibly was due to soil types and inappropriate cultivations. Its pulps are not dark pink color and have a bitter taste. On the other hand, the Tubtim Sayam pummelo trees planted in new vernacular orchards as a monocrop in Pak Panang District, Nakhon Si Thammarat Province, have produced higher fruit quality than those grown in Pattani (Maneepong, 2008; Sethpakdee, 2001). The areas for growing Tubtim Sayam pummelo commercially in Pak Panang District have been expanded rapidly in recent years (Maneepong, 2013). The price of Tubtim

Sayam fruits is also much higher than the prices of other cultivars (Promotion and Development, 2009).

Maneepong (2009) and Wongchana (2010) reported that the soil in Pak Panang District of the Nakorn Si Thammarat Province lacked nitrogen. The lack of nitrogen causes the decrease in plant growth. The symptom is obviously seen on old leaves due to the removal of nitrogen from the old to the developing young leaves (Alva et al., 2001; Koo & Young, 1977). The old leaves, therefore, become yellow with shorter ages from 1-3 years to 6 months and there are only the younger pale yellow leaves on the branches. With more leaves falling, branches become dry and die from the top. This causes poor, low and small citrus fruit production (Osotsapa, 2009; Ritenour et al., 2006). A crucial role of nitrogen is a primary element of amino acid, protein, chlorophyll, enzyme, auxins and cyto-kinins hormones, nucleic acid, alkaloid, and other nitrogen elements like adenosine triphosphate (ATP) and co-enzyme (Alva et al., 2008, 2006; Cantarella et al., 2003; Osotsapa, 2001; Quaggio et al., 2000; Storey and Treeby, 2000; Warren et al., 2000; Feungchan, 1995; Domingo et al., 1992; Hewitt, 1984).

As they have been reported that the nitrogen addition influence of the quality of citrus (Alva et al., 2008; Alva et al., 2006; Ritenour et al., 2006; He et al., 2003; Quaggio et al., 2000; Obreza and Rouse, 1993; Cameron and Dennis, 1986; Dasberg et al., 1983; Sahota and Arora, 1981). Therefore, in this study, the effect of nitrogen on the reproductive growth and fruit quality of Tubtim Sayam pummeloes was investigated. Different levels of nitrogen were applied onto the five year old trees of Tubtim Sayam pummeloes.

RESEARCH METHODOLOGY

1. Plant material

The experiment was conducted on the pummelo cv. Tubtim Sayam trees of 5 years old in Pak Panang District, Nakhon Si Thammarat Province, during January 2013 to February 2014. The study was conducted in Completely Randomized Design (CRD) with 4 replications for each of the three treatments as T1) Pummelo trees receiving non fertilizer, T2) Pummelo trees receiving fertilizer at farmer's rate and T3) Pummelo trees receiving fertilizer at farmer's rate with an additional 40 kg of calcium nitrate per rai.

2. Plant growth measurement

Growth parameters were evaluated as fruit weight (g), fruit width (cm.), fruit height (cm.) and peel thickness (cm.). The fruit weight was measured with digital scales. The fruit width, fruit height and peel thickness were measured using a caliper.

3. TSS, TA, pH and vitamin C measure-ment

TSS was determined with hand refractometer. Total acid (TA) was titrated with sodiumhydroxide to the end point pH 8.2 using a Microprocessor-based. The volume of sodium hydroxide was used to calculate the percentage of total acid by Boland (1995). pH was determined with pH-meter and vitamin C was recorded by putting 2 ml of the pummelo juice into a beaker with 5 ml of extracting solution (metaphosphoric acid-acetic acid solution; titrating it with standardized dye solution 50 mg of dichloro indo phenol sodium salt in 50 ml of distilled water and 42 mg of sodium bicarbonate) of ascorbic acid, concentration calculation according to AOAC (2000) method.

4. Sugar type and limonin measurement

Sugar contents (glucose, fructose, sucrose and total sugar) were measured using high performance liquid chromatography (HPLC). The chromato-graphic analysis was carried out using a Sugar SH1011 (Shodex size 8.0 mm ID x 300 nm) column and the refractive Index detector. The mobile phase consisted of 0.04 N of sulfuric acid, the method was in accordance with AOAC (2000). The pummel juice was filtered through a cellulose acetate syringe filter 0.20 µm, diameter 13 mm and the filtrate was placed in 2-ml Vial and was subsequently injected to HPLC. Limonin contents were measured using high performance liquid chromatography (HPLC) that consisted of variable wave length detector 210 nm, Hypersil ODS 4.0 x 250 mm column, flow rate 1.0 ml/min, injection volume 20 µl. The mobile phase consisted of acetone nitrite/methanol/water (31.8/22.7 /45.5v/v), according to the method of AOAC (2000).

5. Pulp color measurement

Pigment contents (chlorophyll A, B, β-carotene, lycopene) in the pulp of the Tubtim Sayam pummelo was studied by Simple method (Nagata and Yamashita, 1992). All pigments in samples (1 g of peel or pulp) were extracted with acetone and hexane (4:6) at once, then optical density of the supernatant at 453 nm, 505 nm, 645 nm and 663 nm were measured by spectro-photometer at the same time. Anthocyanin contents of the pummelo pulp measured by pH-differential (Giusti and Wrolstad, 2005), they were reported in mg of cyaniding-3-glucoside/100 g fresh weight basis).

6. Statistical analysis

The analysis of variance (ANOVA) was carried out on experimental data. In the case of significance, means were compared with Duncan's new Multiple Range Test (DMRT) by SPSS Software.

RESULTS AND DISCUSSION

Tubtim Sayam pummelo fruits harvested 28 weeks after the flowers bloomed, it was found that those from the three treatments did not show significant difference in their weight, width, height, peel thickness, fresh pulp weight, fresh peel weight, dry pulp weight, dry peel weight, total soluble solid, total acid, and vitamin C (Tables 1, 2, 3). The soil in the experimental plots in Pak Panang District of Nakon Si Thammarat did not lack nitrogen and the leaves had higher concentration of nitrogen than the appropriate level (2.50-3.00 %). Actually their 3.67 % (data not presented) concentration was in the range of an excessive level (3.34-4.41 %) for several cultivars of citruses (Alva et al., 2008, 2006; Gallasch, 2001; Punchaisri and Tonsuwan, 2001; Tucker et al., 1995) which shows toxic signs in swollen peel, thick peel, reduced soluble solid, fruit size and yield (Damrongrak, 2007; Alva et al., 2006; Schumann et al., 2003; Alva et al., 1998; Obreza, 1993; Koo, 1988; Dasberg et al., 1984; Dasberg et al., 1983). Probably excessive nitrogen which normally affects the balance of other nutrients such as phosphorous, potassium, calcium, and zinc (Elhassan et al., 2011; Zekri and Obreza, 2006; Quaggio et al., 2000; Marcelle, 1995; Serna et al., 1992) did not harm the Tubtim Sayam pummeloes because this cultivar had high immunity. However, the trees with high concen-tration of nitrogen in T3 yielded fruits of which juice had the lowest pH that was in agreement with the highest concentration of acid. This level was significantly different from that of T1 and T2. And the trees of T2 had the highest of TSS per TA ratio (39.31) compared with those in the other treatments (27.0-33.3). The result is similar to the previous reports (Liu et al., 2008; Lee and Kader, 2000; He et al., 1999) that high addition of nitrogen could reduce the fruit quality. In addition, it was found that all the three treatments gave same amount of TSS. However, when the types of sugar in the juice was analyzed, it was found that the pummeloes of T2 had the highest amounts of sucrose, glucose, fructose, and total sugar (124.06, 50.61, 16.59 and 191.26 grams per liter), respectively (Table 4), These amounts differed significantly from those found in the pummeloes of T1 and tended to be higher than those found in the pummeloes of T3. It was possible that the nitrogen given in T2 helped the balance of nutrients involved in producing sugar such as phosphorous, potassium, calcium, and zinc (Obreza et al., 2008; Alva et al., 2006; Koo, 1988). On the other hand, the added calcium nitrate of T3 caused the pummeloes trees to absorb excessive nitrogen. T3 produced the lowest amount of limonin (1.99 mg/l) which was significantly different from that of T2 and T1

(2.49 and 3.20 mg/l, respectively) because nitrogen is an important element of triethylamine oxide (TMAO), which can stop the synthesis of limonin found in the inner peel, segment dividers, and seeds by preventing limonoate D-ring lactose hydrolase enzyme from changing non-bitter limonoate A-ring lactone: LARL into bitter limonin (Berhow et al., 2000; Van Beek and Blaakmeer, 1991).

Table 1 Fruit weight, fruit width, fruit height, and peel thickness of Tubtim Sayam pummeloes from T1, T2, and T3 treatments

Treatment	Fruit weight (g)	Fruit width (cm.)	Fruit height (cm.)	Peel thickness (cm.)
T1	1,207.9±169.9	14.6±0.7	16.2±1.27	0.95±0.08
T2	1,147.4±201.3	14.6±1.4	15.7±0.47	1.06±0.07
T3	1,264.9±99.7	14.4±0.9	16.2±0.2	1.15±0.13
CV.	13.47	6.30	4.90	9.40
F-test	ns	ns	ns	ns

[1]Means with different alphabets in the same column show significant difference at $P < 0.05$

Ns = non significant difference

Table 2 Fresh pulp weight, fresh peel weight, dry pulp weight, and dry peel weight of Tubtim Sayam pummeloes from T1, T2, and T3 treatments

Treatment	Fresh pulp weight (g)	Fresh peel weight (g)	Dry pulp weight (g)	Dry peel weight (g)
T1	763.5±97.9	442.4±108.8	105.8±12.2	96.5±5.3
T2	719.3±126.6	427.6±75.4	95.4±11.1	93.9±11.1
T3	816.9±64.4	446.2±34.7	109.7±10.3	104.2±11.5
CV.	12.90	18.00	12.20	19.40
F-test	ns	ns	ns	ns

[1]Means with different alphabets in the same column show significant difference at $P < 0.05$

Ns = non significant difference

Table 3 TSS, TA, TSS/TA, pH, and vitamin C of Tubtim Sayam pummeloes from T1, T2, and T3 treatments

Treatment	TSS (brix)	TA (%)	TSS/TA	pH	Vitamin C (mg/100 ml)
T1	11.0±0.2	0.33±0.02	33.3±0.0[b]	4.45±0.13[a]	57.25±0.21
T2	11.4±0.5	0.29±0.09	39.31±0.0[a]	4.21±0.20[ab]	55.47±0.48
T3	10.8±0.5	0.40±0.07	27.0±0.0[c]	4.00±0.04[b]	57.37±0.47
CV.	3.80	16.80	12.20	3.50	2.20
F-test	ns	ns	**	*	ns

[1]Means with different alphabets in the same column show significant difference at $P < 0.05$

Ns = non significant difference

Table 4 Sugar types and limonin of Tubtim Sayam pummeloes from T1, T2, and T3 treatments

Treatment	Sucrose (g/l)	Glucose (g/l)	Fructose (g/l)	Total sugar (g/l)	Limonin (mg/l)
T1	88.17±1.89[b]	28.39±5.93[b]	8.48±2.61[b]	125.05±8.13[b]	3.20±0.38[c]
T2	124.06±22.98[a]	50.61±7.69[a]	16.59±1.77[a]	191.26±30.63[a]	2.49±0.20[b]
T3	118.37±17.38[a]	45.61±6.29[a]	16.06±0.52[a]	180.04±24.18[a]	1.99±0.00[a]
CV.	15.10	16.00	13.40	13.90	9.70
F-test	**	**	**	**	**

[1]Means with different alphabets in the same column show significant difference at $P < 0.05$

Ns = non significant difference

Concerning the pigments of pummelo pulp, it was found that the amounts of chlorophyll A(0.01-0.04 mg/100 ml) and chlorophyll B(0.01-0.05mg/100 ml) were similar no matter the trees were given nitrogen or not. On the other hand, the pulp of the pummeloes from T3 had the highest amounts of lycopene (2.38 mg/100 ml) and anthocyanin (7.01 mg/100 ml). This result was significantly different from that of T1. These results are differentiated with the reports on the studies of the other fruits. Stefanelli et al. (2010) reported the negative effects of high nitrogen applica-tions on reducing red color or increasing the green background of apples were due to a decrease in both chlorophyll degradation and anthocyanin synthesis in the peel. However, low nitrogen applica-tions in grapes resulted in an increase in total anthocyanin (Hilbert et al., 2003). The pulp of fruits from T1 had the highest amount of β-carotene (0.55 mg/100 ml), which was significantly different from that with nitrogen fertilizer applications (Table 5). The colors in

pummelo pulp came from two groups of pigments, the water soluble group which included all kinds of anthocyanin and the lipid soluble group which included chlorophyll, β-carotene, and lycopene. The mixture of these pigments resulted in the color of the pulp. Anthocyanin, lycopene, and β-carotene, in descending order, contributed to the deepred color of the Tubtim Sayam pummeloes under the cover of the green pigment of chlorophyll. When the fruit entered the middle phase of its growth, it lost the chlorophyll and began to reveal other colors (Tripoli et al., 2007; Siriphanich, 2003; Ortuno et al., 1997; Skrede et al., 1992). At this point the fruit produced phenylalanine ammonialyase (PAL), which is an important enzyme in synthesizing such organic components as phenolic, flavonoid, tannin, lignin, and anthocyanin. The increasing activities of PAL corresponded with the increase in ethylene synthesis which occurred when fruits entered the ripening phase and changed from green color into different colors according to their cultivars (Siriphanich, 2003; Lee, 2002; Frometa and Echazabal, 1988). Internal factors such as fruit age, gene, and external factors such as light, temperature, growth regulators and nutrients had a great impact on the production and activities of PAL (Fukuoka et al., 2014; Louis et al., 2010; Lee, 2002; Manthey et al., 2000; Ortuno et al., 1997; Mazza and Miniati, 1993). Therefore it can be assumed that the application of a large amount of nitrogen in the experimental plots in Pak Panang District of the Nakon Si Thammarat Province and the amount of nitrogen in the leaves which was higher than the appropriate level were favourable factors for PAL production and activities which increased anthocyanin and lycopene, making the pulp have a deep red color.

Table 5 Pigments in pulp of Tubtim Sayam pummeloes from T1, T2, and T3 treatments

Treatment	Chlorophyll A (mg/100 ml)	chlorophyll B (mg/100 ml)	Lycopene (mg/100 ml)	β-carotene (mg/100 ml)	Anthocyanin (mg/100 ml)
T1	0.04±0.00	0.05±0.00	1.53±0.09[b]	0.55±0.15[a]	4.76±0.53[b]
T2	0.02±0.00	0.01±0.00	2.16±0.10[a]	0.37±0.07[b]	6.76±0.02[a]
T3	0.01±0.00	0.01±0.00	2.38±0.19[a]	0.29±0.04[b]	7.01±0.57[a]
CV.	13.17	16.56	8.40	4.50	7.30
F-test	ns	ns	**	**	**

[1]Means with different alphabets in the same column show significant difference at $P < 0.05$

Ns = non significant difference

CONCLUSIONS

The Tubtim Sayam pummeloes fruits from the Tubtim Sayam pummeloes trees that received the fertilizer at farmer's rate were the best quality because of their highest amount of total sugar. Tubtim Sayam pummeloes tree after receiving fertilizer at farmer's rate with additional calcium nitrate gave the fruits with the highest amount of lycopene and anthocyanin which are sources of antioxidants and that fruit had the lowest amount of limonin which are source of bitter flavour.

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