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Verification of nutrients standards for diagnosis of Yuhebao lychee (*Litchi chinensis* Sonn.)

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The steady production of Yuhebao lychee (*Litchi chinensis* Sonn.) is involved in accurate fertilization management. The critical period of nutrient requirement for Yuhebao lychee is during January to June every year in Taiwan because the period is from flowering to fruiting stage. It would serve the references of fertilization if there is an accurate standard for nutrient management of Yuhebao lychee. This study was conducted to establish the accurate standard of sampling period and location for nutrient diagnosis of Yuhebao lychee by analyzing essential nutrient elements of leaves from pre-anthesis to harvest stage. The leaves were sampled every month on middle of January to June for the estimation of leaf nutrients by inductively coupled plasma spectrophotometer (ICP). The results revealed that the appropriate sampling position and period of leaves should be from the leaves below flower spike during the flowering period. On the other hand, the mature leaves from either the branch of flowering or non-flowering terminal for nutrient management of Yuhebao lychee is recommended.

Key words: Agricultural management, climate variations, lychee, nutrient diagnosis.

INTRODUCTION

Lychee is one of the economically cultivated fruit trees in many countries. Yuhebao is one of the important lychee varieties that occupy a broad area in Taiwan. Especially the climate is characterized by middle rainfall (about 1,968 mm), and the average temperature is 11-13 ℃ in winter to 25-32 ℃ in summer. The suitable soil texture for Yuhebao lychee is sandy to sandy loam and soil pH are from 4.5 to 6.0. Nutrients management for another lychee cultivars were reported (Yao et al., 2020) except Yuhebao lychee. All of the N, K, Ca, Zn, and S will maintain fruit development when they are taken up abundantly by the plant during fruit enlargement period, and partial P, Mg, Mo and B are translocated from leaves of the first and second vegetative flushes (Yao et al., 2017b). When the nutrients are deficient, visual symptoms of N, K, Mg, Fe and Zn in lychee leaves were apparent (Singh et al., 2019), and the production of lychee were influenced. Steady production of lychee plants is involved in accurate nutrients diagnosis and management. Sufficient nutrition of lychee is important, however, fruit yield is significantly suppressed by excessive supplement (e.g. boron) in spite of no visual symptoms that are found in lychee plants in the orchard (Wojcik and Wojcik, 2003). Hence, establishment of nutrients standard on leaf and sampling period are important procedure to assess the nutrients abundance

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of lychee plants. The variation of nutrients concentration in lychee leaves changed following the maturity degree of leaf and getting stability at the maturity stage (Yang et al., 2015). Lychee grows perennially, and foliar nutrient concentration varies with the different growth stage (Luo et al., 2019; Yang et al., 2016). In the past, many attempts were proceeded for this research, however, accurate leaves sampling period and location for nutrients diagnosis of Yuhebao lychee were established only on N, P, K, Ca, Mg, Fe, Mn (Basavaraj et al., 2017; Singh et al., 2019). On the other hand, it is difficult to establish various leaf nutrient norms due to divergences of soil type, diagnosis time, and the methods to standardize the norms. In fact, a tentative nutrition program, illustrating the fertilizer choices based on phenological stage of lychee, was suggested in Australia (Martínez-Bolaños, 2020). Nowaday, appropriate fertilization by leaf nutrients diagnosis to the phytoprotection (e.g. greater tree size and bearing canopy) of lychee tree were preceded in Australia, India, South Africa, and the United State (de Villiers and Joubert, 2010). For example, after nutrients diagnosis, foliar application of 3% $CaCl_2$+1.5% borax ($Na_2B_4O_7 \cdot 10H_2O$) not only increases single fruit weight but also enhances the contents of total sugars, reducing sugars and nonreducing sugars in lychee fruits (cv. Gola) in Pakistan (Haq et al., 2013). Spraying 0.2% or 0.5% $CaCl_2$ after full blossom does not effectively enhance structural Ca in litchi pericarp, however, generally reduces fruit cracking rate in lychee (Huang et al., 2008). The critical period of nutrient management for Yuhebao lychee is during January to June every year in Taiwan. It would be serve the references of fertilization if there is an accurate standard for nutrient management of Yuhebao lychee. For above-mentioned reasons, this study is comprehensively made to standardize the sampling period and location at first for the nutrient diagnosis of Yuhebao lychee. Advanced experiment was proceeded at the Fanliao, Taiwan. After nutrients diagnosis on February, 2019, fertilizers were supplied by foliar fertilization to investigate the inhibition of fruits dropping at fruit-setting stage and fruit quality at harvesting stage. It is available for the phytoprotection and steady production of Yuhebao lychee.

MATERIALS AND METHODS

Chosen orchards site and experimental scheme

12 Yuhebao lychee orchards in Taiwan were chosen for this experiment. Three orchards in Henchun, one in Mancho, one in Fangliao, two in Gaosu Pingtung and three orchards in Dashu, two in Chisan, Kaohsiung. The leaves of Yuhebao lychees were sampled from steady-produced, full bearing Yuhebao lychee orchards. The location, geographic measurements, age of orchard, spacing, the properties of soil, soil nutrient status were showed in Table 1; 8 to 13 years old of orchards were chosen. The soil texture of orchards was from loam to sandy loam. The electrical conductivity (EC) was 0.18 to 0.25 mS cm^{-1} and pH was 4.5 to 6.3. The soil nutrients phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn) were estimated to be abundant, respectively.

About ten trees were chosen and the branches were tagged. The 4th pairs of leaves from the top of the non-flowering (NFB) and flowering (FB) branches were sampled, respectively. The leaves were sampled from every middle of January to June. As a result of mature leaves of lychee is the most stable position for nutrients diagnosis (Menzel et al., 1988).

This study was conducted to sample the leaves for analysis of nutrients from anthesis and non-anthesis branches. A mixed sample of twenty five leaves from every direction was sampled from terminal 4 to 5 position in every distinguishment. Leaves of lychee were collected and washed with distilled water. The obtained datas were statistically analyzed with variance analysis and Duncan test (at 0.05% probability), for wich SAS 9.1.3 (SAS Institute Inc., Cary, NC, USA) was employed.

Practically application of nutrient diagnosis standard on Yuhebao lychee

Following, the nutrients condition was analyzed for the diagnosis of lychee before anthesis stage at the orchard of Fangliao, Pingtung (February, 2020). The nutrient condition was Ca, Cu and B deficiency when comparing with the standard in Table 5. And then the deficient nutrients were supplied to evaluate the effect of phytoprotection (Inhibition of fruits dropping). Hence, the three treatments of 0.2% $CaCl_2$, 0.2% $CuSO_4$ and 0.1% H_3BO_3 were designed, respectively, and another treatment was the mixture of three designed by front three solution. The non-sprayed treatment was designed, too. Three Yuhebao lychee trees were chosen for every treatment and the prepared solution were sprayed respectively from March, 2020 for three times, two weeks interval. And the percentage of fruit-dropping was evaluated during fruit-setting stage.

Nutrient analysis of Yuhebao lychee leaves

The sampling position for nutrient analyzing was the mature leaves below flowering branchs. The leaf samples were collected and washed by distilled water, and then oven-dried at 70 ºC for 1 day. The oven-dried samples were then grinded to obtain homogenous finer samples. Sulfuric acid was added at 350 ºC for the complete digestion and then K, Ca, Mg, Fe, Mn, Zn, Cu were determined by ICP (Inductively Coupled Plasma) spectrophotometer. The oven-dried samples were analyzed for N using elemental analyzer (Gholizadeh et al., 2009), P by the method of molybdenum blue (Shtangeeva et al., 2017).

Data analysis method

The obtained datas were statistically analyzed with variance analysis and Duncan test (at 0.05% probability), for which SAS 9.1.3 (SAS Institute Inc., Cary, NC, USA) was employed.

RESULTS

Establishment of nutrient diagnosis standard for Yuhebao lychee

Leaf analysis in different months for the nitrogen

Table 1. The location, land form, age of orchard, spacing, soil properties and soil nutrient status of Yehebao lychee orchards.

Location	Landform	Age of orchards (years)	Spacing (cm×cm)	Soil properties				Available Nutrients			
				Texture	pH	EC (mS/cm)	OM (%)	P (mg/kg)	K (mg/kg)	Ca (mg/kg)	Mg (mg/kg)
Henchun 1*	Plain	10	8 × 10	Loam	5.7	0.21	2.11	35	70	686	65
Henchun 2	Slopland	9	8 × 8	Loam	5.0	0.12	1.55	18	62	533	53
Henchun 3	Plain	11	5 × 8	Sandy loam	5.6	0.17	2.23	41	80	778	76
Mancho	Plain	8	8 × 10	Sandy loam	6.3	0.25	2.50	52	91	953	83
Fangliao	Slopland	13	10 × 10	Sandy loam	4.5	0.11	1.0	25	42	205	21
Gaosu 1	Plain	12	5 × 8	Loam	6.0	0.25	1.24	56	74	598	43
Gaosu 2	Slopeland	9	8 × 10	Loam	4.6	0.17	1.08	23	49	402	38

*Three orchards located in Henchun, one in Mancho, one in Fangliao and two in Gaosu, Pingtung.

Table 2. The variety of nutrients concentration in the leaves of non-flowering branch (NFB*) from January to June.

Month	Nutrient								
	N (%)	P (%)	K (%)	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Mn (mg kg^{-1})	Fe (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})
January	0.90^{b}	0.08^{b}	0.90^{a}	6725^{ab}	2384^{b}	123.1^{b}	343.0c	43.2^{a}	86.9^{ab}
February	1.02^{a}	0.14^{b}	0.92^{a}	7016^{a}	2932^{ab}	362.1^{a}	563.8^{ab}	43.0^{a}	90.0^{ab}
March	1.14^{a}	0.14^{b}	0.92^{a}	7383^{a}	2716^{ab}	258.6^{ab}	448.6^{b}	42.8^{a}	89.7^{ab}
April	1.19^{a}	0.19^{a}	0.86^{a}	8848^{a}	3704^{a}	345.5^{a}	593.85^{ab}	35.9^{a}	225.5^{a}
May	1.14^{a}	0.12^{b}	0.75^{a}	6628^{ab}	3372^{a}	291.4^{ab}	1009.2^{a}	48.3^{a}	271.1^{a}
June	1.06^{a}	0.15^{ab}	0.92^{a}	4914^{b}	2278^{b}	203.7^{ab}	465.5^{b}	33.5^{a}	51.48^{b}

*Non-flowering of branch on Yuherpao lychee.

(N) content showed the slightly increase from January to April and decrease from April to June on NFB (Table 2) and the increase from January to May and decrease from May to June on FB (Table 3). The most significantly stable period falls between March to May with N nutrient, and follow by decreasing of N concentration. Leaf phosphorus was maintaining steady and increasing in April and then decreasing continuously till June. The significant stabilization phosphorus (P) contents fall during January and March. Leaf potassium (K) curve showed that the significant steady period during January and March no matter in the NFB or FB and then decrease at the subsequent months. Leaf calcium (Ca) concentration continuously increased from January to April. However, Ca concentrations decreased between May and June which was apparently low. Magnesium (Mg) concentration in leaves showed a steady period between January and March no matter in the NFB or FB, however, the continuous stability is maintained on NFB and concentration in FB is large alteration during May and June.

Leaf copper showed stable variation from January to June in the leaf of NFB (Table 2) and FB (Table 3), however, there is slightly increase in

Table 3. The variety of nutrients concentration in the leaves of flowering branch (FB*) from January to June.

Month	Nutrient								
	N (%)	P (%)	K (%)	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Mn (mg kg^{-1})	Fe (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})
January	0.93^{b}	0.15^{a}	0.86^{a}	5186^{b}	3545^{a}	135.5^{a}	184.3^{b}	23.3^{ab}	42.3^{ab}
February	1.07^{ab}	0.15^{a}	0.86^{a}	7006^{ab}	3149^{a}	191.9^{a}	272.8^{ab}	21.5^{ab}	45.0^{ab}
March	1.18^{a}	0.13^{a}	0.78^{a}	7945^{a}	3076^{ab}	145.7^{a}	231.6^{ab}	21.4^{ab}	44.9^{ab}
April	1.14^{a}	0.18^{a}	0.67^{a}	7063^{ab}	4387^{a}	196.2^{a}	290.1^{ab}	12.1^{b}	109.1^{a}
May	1.17^{a}	0.12^{a}	0.52^{a}	7586^{a}	4800^{a}	177.8^{a}	453.5^{a}	28.4^{a}	118.6^{a}
June	0.92^{b}	0.15^{a}	0.86^{a}	5569^{b}	2604^{b}	113.9^{a}	203.6^{b}	13.1^{b}	21.4^{b}

*flowering of branch on Yuherpao lychee.

the leaf Cu of FB on May. The stable period of copper (Cu) was observed between January to June. The stabilization of leaf Cu values was observed between January to June. Foliar zinc (Zn) concentration also showed similar trend with copper for steady period during February and May. The leaf manganese (Mn) concentration had a similar variation in the leaf of NFB and FB which were steady except they are lower in the leaf concentration on March. However, the steady period of iron (Fe) falls during January to April and dramatic increase on May, and decrease on June. The final establishment of Yuhebao lychee for nutrients standard was listed in Table 4. The suitable sampling period was from February to March, and appropriate sampling position was the mature leaves either below non-flower branch or flower branch.

Practically application of lychee lychee nutrient diagnosis standard

After nutrients diagnosis, the supplement of deficient nutrients for the inhibition of fruit-dropping at Henchun and Fangliao showed in Table 5. In the orchard of Henchung, fruit-dropping was significantly higher on the district of check than that of treatment. The treatment of $CuCl_2$ and H_3BO_3 were higher than that of $CaCl_2$. However, the treatment of mixed solution was not high for the inhibition of fruit-dropping. In the orchard of Fangliao, the fruit-dropping was highest in the district of check. The inhibition of fruit-dropping was comparatively good on the treatments of $CaCl_2$ and H_3BO_3 than that of $CuCl_2$.

DISCUSSION

In this study, the steady period for foliar N and K falls during March to May and for P in January to March in the leaves of NFB and FB which were earlier than the Malhotra et al. (2018), who reported steady period for leaves N, P and K of Tai So, Haak Yip and Wai Chee during May and August in subtropical Australia. The present study was preceded at tropical district of south Taiwan. The reproductive growth period at this district for Yuherpou lychee was shorter with 100-150 days compared to 150-200 days at the subtropical regions Queensland, Australia (Olesena et al., 2013). The different climate and short reproductive period in south Taiwan might be the reason reaching early stabilization period of leaf N, P and K in this research. N and P decreased from March (flowering stage) towards June (postharvesting stage) might be the synthesis and formation of carbohydrate in fruits and causing the decrease of two nutrients (Yang et al., 2014). Leaf K was steady from January to March and decreased from March to May. It might be due to the transporting of K to fruits (Xu et al., 2020). Malhotra et al. (2018) reported levels of Ca and Mg rose during flowering and fruit growth to reach maximum values and decreased after harvest to minimum values. The steadiest period for leaf Ca and Mg concentrations in Yuhebao lychee was during January to March compared to April to June in the present research.

Zavalloni et al. (2001) observed that dilatory mobility of Ca and Mg cause that they were difficult to reach the stable periods in apple.Hence, Ca and Mg might reach steady values after their mobility to other tissues (Singh et al., 2019).

Table 4. The nutritions concentration and standard deviation of leaves from January to June.

Leaves position	Nutrients concentration								
	N (%)	P (%)	K (%)	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Mn (mg kg^{-1})	Fe (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})
January									
NFB	0.9±0.3	0.1±0.04	0.8±0.2	0.5±0.1	0.21±0.0	161±57	199±61	22±5	40±12
FB	0.9±0.3	0.1±0.04	0.7±0.2	0.4±0.2	0.21±0.0	166±66	190±66	21±6	39±12
February									
NFB	1.0±0.3	0.1±0.0	0.8±0.2	0.7±0.2	0.3±0.1	190±99	263±64	21±7	99±11
FB	1.0±0.4	0.1±0.0	0.8±0.3	0.7±0.2	0.3±0.1	169±88	250±76	21±6	90±12
March									
NFB	1.2±0.3	0.1±0.0	0.8±0.2	0.8±0.3	0.3±0.1	137±89	220±79	21±5	104±27
FB	1.1±0.4	0.1±0.0	0.8±0.2	0.8±0.3	0.3±0.1	124±83	205±80	22±6	96±25
April									
NFB	0.9±0.5	0.1±0.0	0.6±0.3	0.9±0.5	0.4±0.2	170±92	238±124	10±6	92±48
FB	0.9±0.4	0.1±0.0	0.5±0.2	0.9±0.4	0.4±0.2	164±91	235±103	10±6	90±45
May									
NFB	0.9±0.5	0.1±0.0	0.4±0.2	0.6±0.3	0.4±0.2	157±87	367±194	24±12	103±56
FB	0.9±0.3	0.1±0.0	0.4±0.2	0.6±0.2	0.4±0.2	150±88	367±150	23±12	99±56
June									
NFB	0.7±0.3	0.1±0.0	0.7±0.3	0.5±0.2	0.2±0.1	93±66	160±77	12±5	18±9
FB	0.7±0.3	0.1±0.0	0.7±0.3	0.5±0.2	0.2±0.1	97±70	161±76	11±7	17±10

Leaves of non-flowering branch FB: Leaf of flowering branch.

Table 5. Effect of supplement of deficient elements by foliar Fertilization on the percentage of fruit dropping for Yuhebao lychee.

Treatments	Fruit dropping (%)	
	Henchung	Fangliao
*CK	63.7[a]	50.8[a]
0.2% $CaCl_2$	57.8[a]	35.5[b]
0.2% $CuSO_4$	22.7[b]	50.0[a]
0.1% H_3BO_3	24.1[b]	29.0[b]
**Mixed solution	31.8[b]	46.5[a]

*CK: Check; **Mixed solution: 0.2% $CaCl_2$+0.2%$CuSO_4$+0.1%H_3BO_3.

This study showed that the stable period of Cu and Zn in leaf was between January to June and Zn between February and April, respectively. Xia et al. (2020) stated that the absorption and transportation of Zn in fruit plants were involved with the presence of Cu. Saini et al. (2019) reported the steady period of leaf Fe and Mn concentration in lychee occurred during flowering to fruit-setting stage. They suggested that the stable period of foliar Fe and Mn concentrations at anthesis stage (about February to March in lychee) followed by stable increase till harvesting stage in lychee leaves (Bakshi et al., 2013). This study showed the steady period of foliar Fe and Mn concentration occurred between January and April and January to June, respectively. The differences of Fe and Mn concentrations might be the ability of different cultivars to accumulate these elements. Onwuka and Mang (2018) stated that the seasonal variation in micronutrient status is mainly influenced by crop load factor confirming that the stabilization period observed in our study could be the best time for leaf sampling in Yuhebao lychee. Comprehensively evaluation of these estimated elements, the results showed that the mature leaves collected under FB or the mature leaves of NFB position during February and March is the best suitable time for foliar nutrient analysis in Yuhebao lychee grown in the South Taiwan. And the reference standard of nutrients is shown in Table 4. Commonly, the calcium in the soil of Henchun is sufficient (above 500 mg/kg); however, the absorption of calcium by Yuhebao lychee was insufficient. It may be calcium is unavailable in the soil. On the other hand, the reason why insufficiency of boron and copper in Yuhebao lychee maybe the requirement of microelements for physiological need during anthesis to fruit-setting duration. Soil pH in the soil of Fanglio is below 4.5 (data not shown). Commonly, calcium and boron in the strongly acid soil are insufficient. Hence, the supplement of $CaCl_2$ and H_3BO_3 were both beneficial for phytoprotection (e.g. inhibition of fruit-dropping). On the other hand, the supplement of $CuCl_2$ was more favorable for the inhibition of fruit-dropping than that of check.

Conclusion

The study showed that the overlap stable period of nine elements was occurred between February to March, that is, the sampling period on leaf nutrient status of Yuhebao lychee reveals that the most appropriate leaves sampling period and location was between February to March. No matter what the mature leaves under flower of FB or the mature leaves of NFB about the sampling location. By way of nutrients diagnosis during the appropriate sampling period and position, nutrients deficiency can be verified, and then supplement of the deficient nutrients will make the quality of Yuhebao lychee get better. However, this research is the first result; it should take years of data to get a reliable result.

CONFLICTS OF INTERESTS

The authors have not declared any conflicts of interests.

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