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Correlations Between Leaf N, P, K, Ca and Fe Levels and The Production of Metabolites in Torbangun *(Coleus amboinicus* Lour.)

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Abstract

Torbangun (*Coleus amboinicus* Lour) is a tropical plant from the Lamiaceae family. Torbangun leaves have a distinct aroma. Torbangun leaves have been traditionally used as a medicinal plant in North Sumatra, Indonesia. Bataknese lactating women in North Sumatra consumed torbangun leaves after giving birth with beliefs that it could increase their breast milk production (a lactagogue), and that the leaves can function as anti-fungal and/or anti-bacterial, analgesic, to reduce blood cholesterol, and clean the human uterus. However, scientific evidence on this traditional herb is limited. This research was conducted to: (1) determine the leaf N, P, K, Ca and Fe in different position along the plant and the leaf age as to diagnose the secondary metabolites content of torbangun leaves, and (2) determine the relationship between N, P, K, Ca and Fe leaf concentrations with their metabolite production. The results of the study showed that: (1) the best leaf position and age to determine of the need of N, P, K, Ca and Fe nutrients are on the 3rd leaf position of 5 months-old plant (2) There was a positive correlation between leaf K concentrations with shoot dry weight, Ca with PAL activity, and Fe with total saponins on the 3rd leaf position of 5 months-old plant.

Keywords: *Coleus amboinicus* Lour, torbangun, nutrient, correlation test, secondary metabolites.

Introduction

Torbangun (*Coleus amboinicus* Lour.) is a tropical plant from the Lamiaceae family. Torbangun leaves have a distinct aroma and have been used as a medicinal plant in North Sumatra, Indonesia (Ekawati et al., 2013). Bataknese lactating women in North Sumatra traditionally consumed torbangun leaves after birth with beliefs that it could increase their breast milk production (Damanik et al., 2001; Damanik et al., 2004; Damanik et al., 2006; Damanik, 2008). In addition torbangun leaves have anti-fungal and/or anti-bacterial (Khattak et al., 2013; Khattak et al., 2013a) and analgesic properties (Alfitra et al., 2010; Devi et al., 2010), can reduce blood cholesterol (Andriani et al., 2012); and clean the human uterus (Damanik et al., 2001, Damanik et al., 2004; Damanik, 2008). The potential of torbangun leaves as lactagogue were indicated by their saponins, flavonoids, polyphenols content that can increase breastfeeding hormones, such as prolactin and oxytocin (Damanik et al., 2006). Consumption of torbangun leaves significantly increased mineral contents in breast milk, including K, Ca, Fe, Zn, Mg (Damanik, 2005). The study of torbangun organic cultivation showed that application of organic fertilizer increased shoot production on torbangun leaves. Application of 12.3 t ha^{-1} cow manure + 1.5 t ha^{-1} rock phosphate + 5.5 t ha^{-1} rice-hull ash to torbangun resulted in a 57.33% higher shoot dry weight per ha and higher metabolite production: total phenolic 12.06%, anthocyanin 41.73% higher than without fertilizing ($P > 0.05$) (Ekawati et al., 2013). However, studies on of torbangun cultures are limited, and their effects on secondary metabolite production and their scientific evidence on health have not been well documented. This research was conducted to: (1) determine the correlations of leaf N, P, K, Ca and Fe of different positions along the plant and plant age to diagnose the secondary metabolites production in torbangun leaves, and (2) determine the relationship between N, P, K, Ca and Fe leaf concentrations with torbangun metabolite production.

Materials and Methods

Experimental Site

The research was conducted between January -

March 2014 in Mulyaharja village (6°36'42.1' S, 106°47'29.2' E), South Bogor District, Bogor, West Java. Plants were grown under shade structures (paranet) with shade intensity of 55%. Analysis of metabolite and leaf nutrient concentrations were conducted at Plant Analysis and Chromatography, Spectrophotometry, Post-Harvest Laboratory, Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University.

Plant materials

This study used 2-month-old RD torbangun accession plants and 15 tons ha^{-1} of chicken manures. Materials used for chemical analysis were analytical grades of methanol, folin-ciocalteu reagent, potassium acetate, acetic acid, sulphuric acid, protein buffer, distilled water and saponins Merck Co. Tools used were scales, oven, hand sprayer, camera (Canon 1100D), laboratory equipment for chemical analysis includes a Shimadzu UV-1800 Spectrophotometer (Japan) associated with UV 2.34 probes for spectrophotometer analysis, Centrifuge Heraeus labofuge-400R, Eyela water bath SB-24 for incubation of solution extracts.

Secondary metabolites analysis

Secondary metabolite and leaf nutrient analysis were conducted on 10 plant samples by harvesting five fully- expanded leaves on the 1st, 3rd, and 5th leaf position from the shoot. The leaf samples were collected once a month for when plants were three, four and five-month-old, and all analysis were repeated three times. Secondary metabolites analysed were *phenylalanine ammonialyase* enzyme activity (PAL) using Dangcham et al. (2008) method, anthocyanin (Sims and Gamon, 2002), total flavonoids of Chang et al. (2002) and total saponins (using modified Fathonah and Sugiyarto (2009) method). Analysis of leaf N concentration used Kjeldahl, method P concentration using Shimadzu UV-1800 spectrophotometer, and the K, Ca and Fe concentration using Atomic Absorption Spectrophotometry Agilent 240 FS AA according to Apriyanto et al., (1989) method.

The correlations between leaf N, P, K, Ca or Fe levels and the production of metabolites were conducted using a simple linear correlation analysis. Based on correlation testing, the concentration of leaf nutrient that have a high positive correlation value and most consistently positioned on the leaves of the same age will be set as torbangun leaves sample using simple linear correlation analyses are as follows:

$$rxy = \frac{n\Sigma X_i Y_i - (\Sigma X_{i)} (\Sigma Y)_i}{\sqrt{[n\Sigma X_i^2 - (\Sigma^{2]} [n\Sigma Y]_i^2 - (Y_{i)}^2}}$$

Correlation value (r) indicates the strength of the linear correlation is in the interval $-1 \leq r \leq 1$. Signs - and + showed the positive or negative correlations.

Results and Discussion

Leaf Nitrogen (*N*)

Leaf N levels varied with leaf positions within the plant (Figure 1). When plants were three-month-old the N levels of the 1st and 3rd leaves were similar, but higher than those of the 5th leaf ($P < 0.05$) (Figure 1). When plants were four-month-old, the N levels of the 1st leaves were higher by 0.54 and 0.79%, respectively, than those of the 3rd and 5th leaves (Figure 1). When plants were five-month-old, the N levels of the 1st leaves were higher by 0.26 and 1.04%, respectively, than those of the 3rd and 5th leaves (Figure 1).

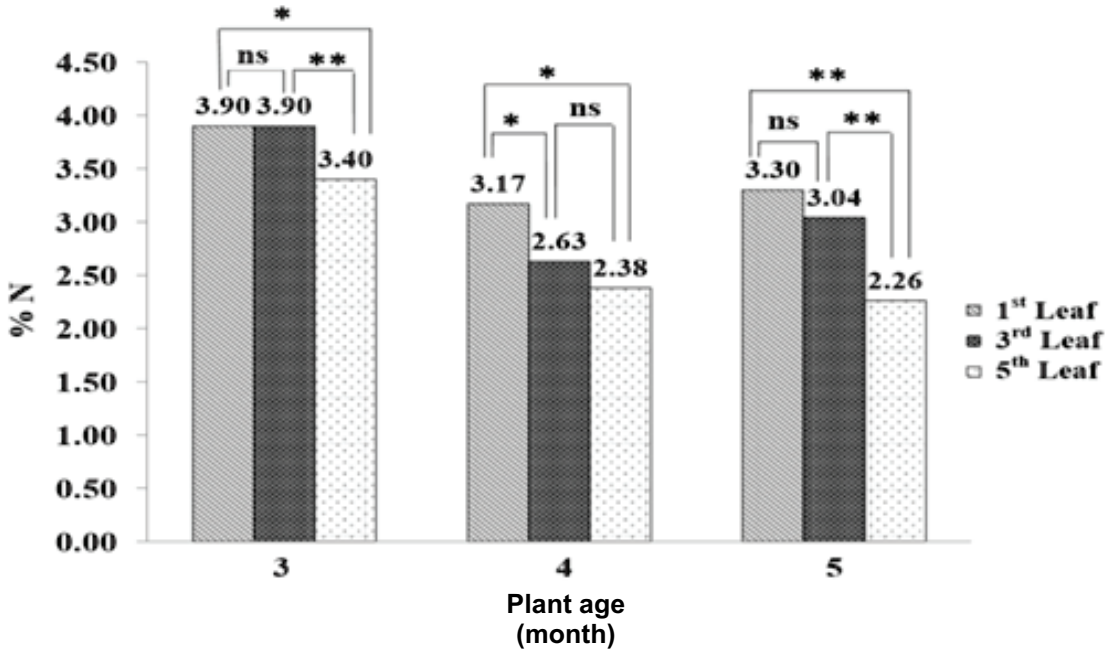

Figure 1. Nitrogen levels of the 1st, 3rd, or 5th leaves of the three, four and five-month-old torbangun plants.

Leaf Phosphorus (*P*)

Similar to N, P leaf levels varied with leaf positions within the plant (Figure 2). When plants were three-month-old the P levels of the 1st and 3rd leaves were similar, but the P level of the 1st leaves were significantly (P <0.05) higher than those of the 5th leaves (Figure 2). When plants were four-month-old the P levels of the 1st leaves were higher than those of the 3rd and the 5th leaves, whereas the P levels of the 3rd and the 5th leaves were similar (Figure 2). When plants were five-month-old, the P levels of the 1st leaves were significantly higher than that of the 3rd (P <0.01) and the 5th leaves (P <0.01) (Figure 2).

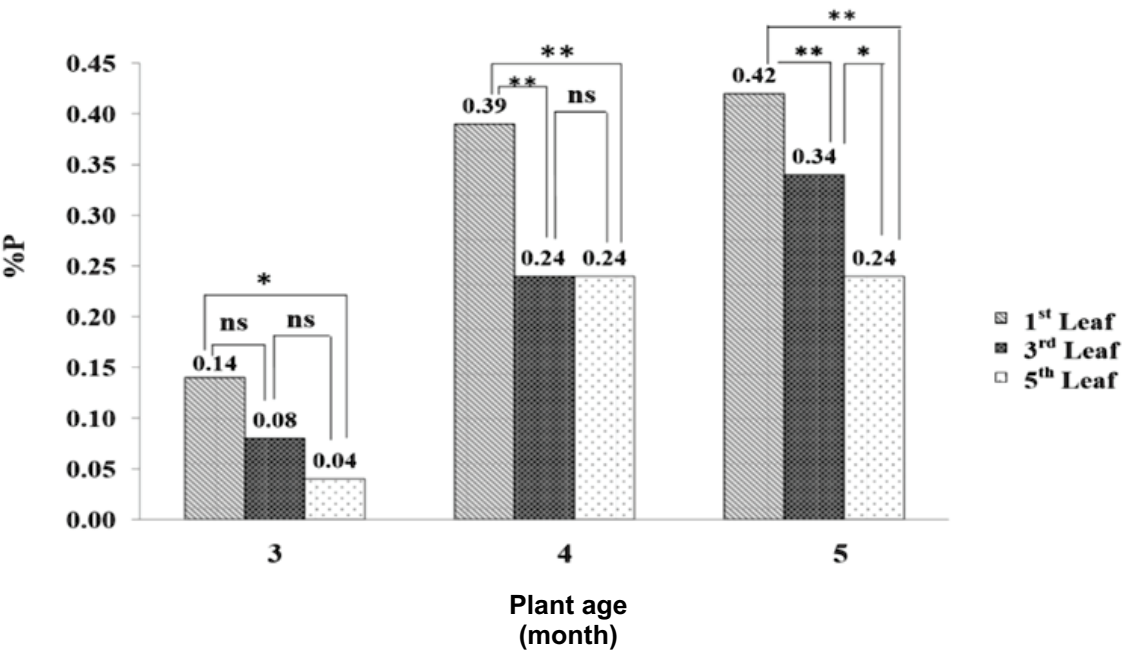

Figure 2. The P levels of the 1st, 3rd, or 5th leaves of the three, four and five-month-old torbangun.

Potassium (*K*)

Leaf K levels were affected by the leaves positions when plants were three-month-old only (Figure 3). K levels of the 1st leaves were not significantly different from those of the 3rd leaves, but were 0.97% higher than the 5th leaves (Figure 3).

Calcium (*Ca*)

Leaf Ca levels were the highest in the 5th leaf (Figure 4) and were significantly higher than those in the 1st leaf when plants were three ($P < 0.01$) and five-month-old ($P < 0.01$) (Figure 4).

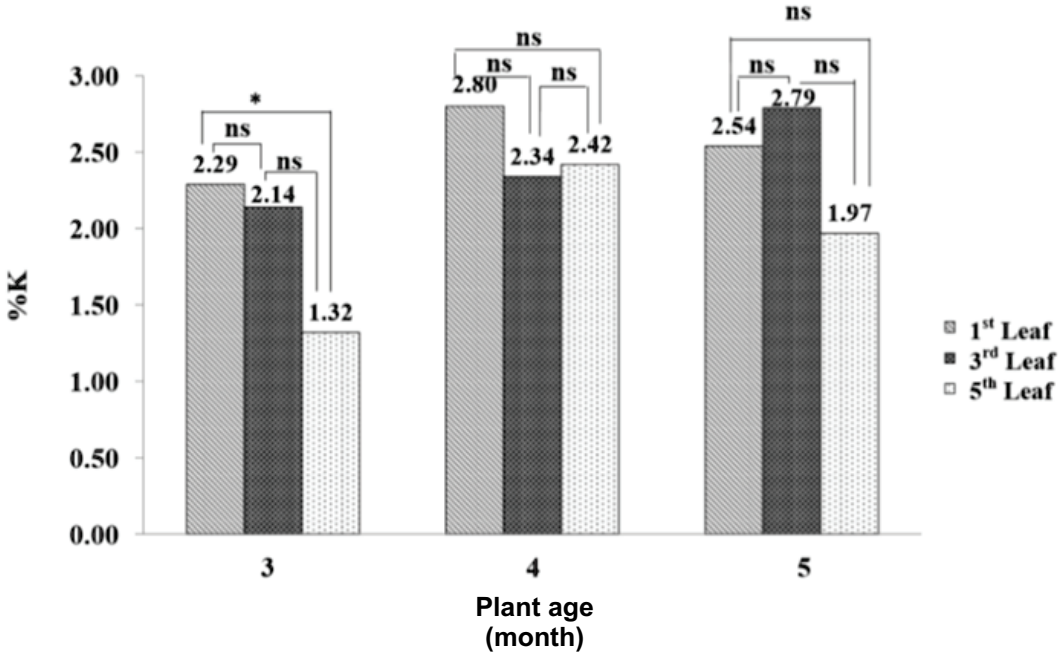

Figure 3. K levels of the 1st, 3rd, or 5th leaf of the three, four and five-month-old torbangun plants.

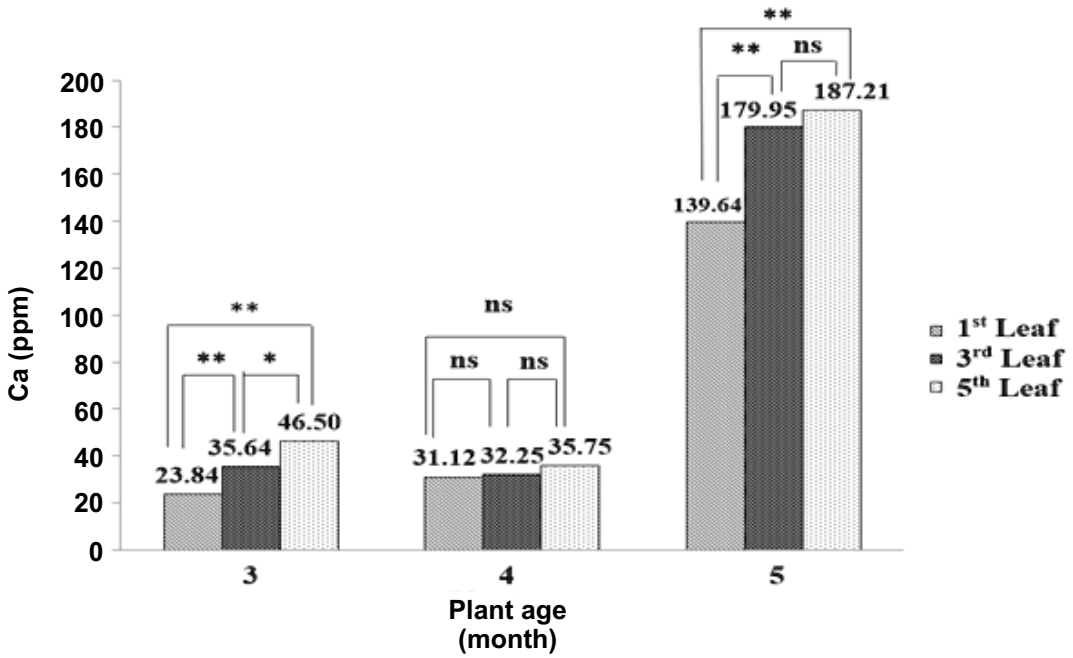

Figure 4. The Ca levels of the 1st, 3rd, or 5th leaf of the three, four and five-month-old torbangun.

Leaf Iron (*Fe*)

The Fe leaves concentrations were not affected by the leaf position in all plant ages (P >0.05, Figure 5).

The Content of Secondary Metabolites on the 1st, 3rd and 5th Leaf Position

PAL activity

Leaf PAL enzyme activities (indicated by the amount of SAS protein in the leaves) of different leaf positions were similar when plants were three and four-month old (Figure 6). However, when plants were five-month-old, the 5th leaf had a significantly higher PAL enzyme activity than the other leaves (P >0.05 and P <0.05, respectively) (Figure 6).

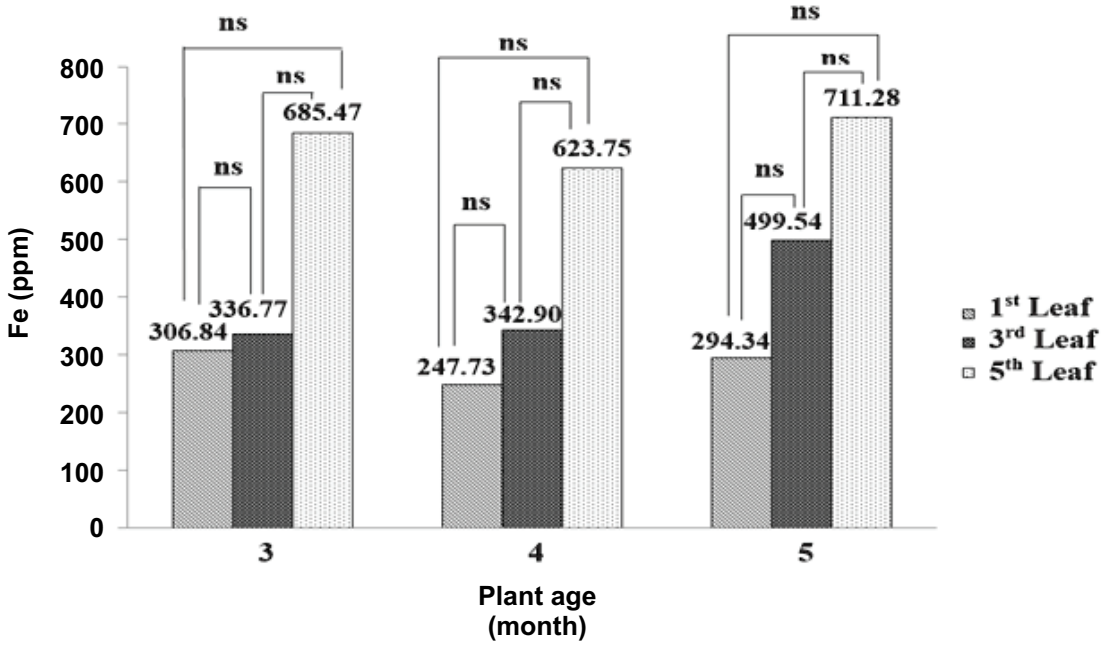

Figure 5. The Fe levels of the 1st, 3rd, or 5th leaf of the three, four and five-month-old torbangun.

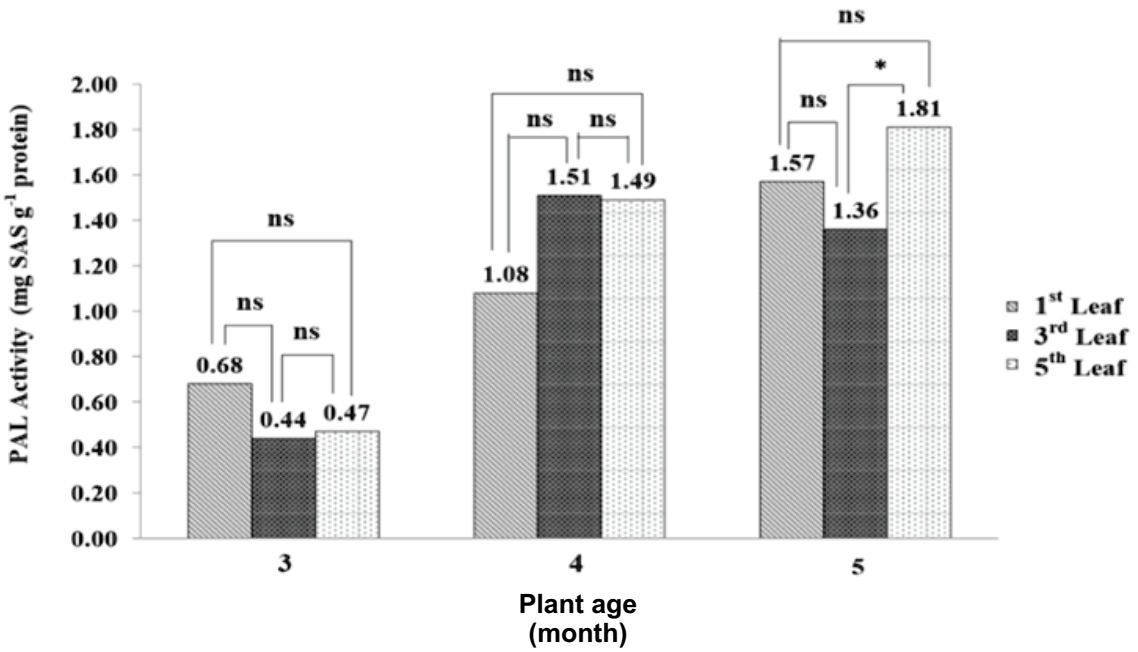

Figure 6. The PAL (*Phenylalanine ammonia-lyase*) activities of the 1st, 3rd, or 5th leaf of the three, four and five-month-old torbangun plants.

Anthocyanin

Anthocyanins levels of the 1st, 3rd and 5th leaves of all plant ages were not significantly different (Figure 7). The leaf anthocyanin levels of the five-month-old-plants increased by six to seven times compared to those of three-old-month plants (Figure 7).

Flavonoids

The total of flavonoids concentrations were not affected by the leaf positions in all plant ages (P >0.05, Figure 8). The leaf total flavonoids increased with the increase of plant ages from three to five month (Figure 8).

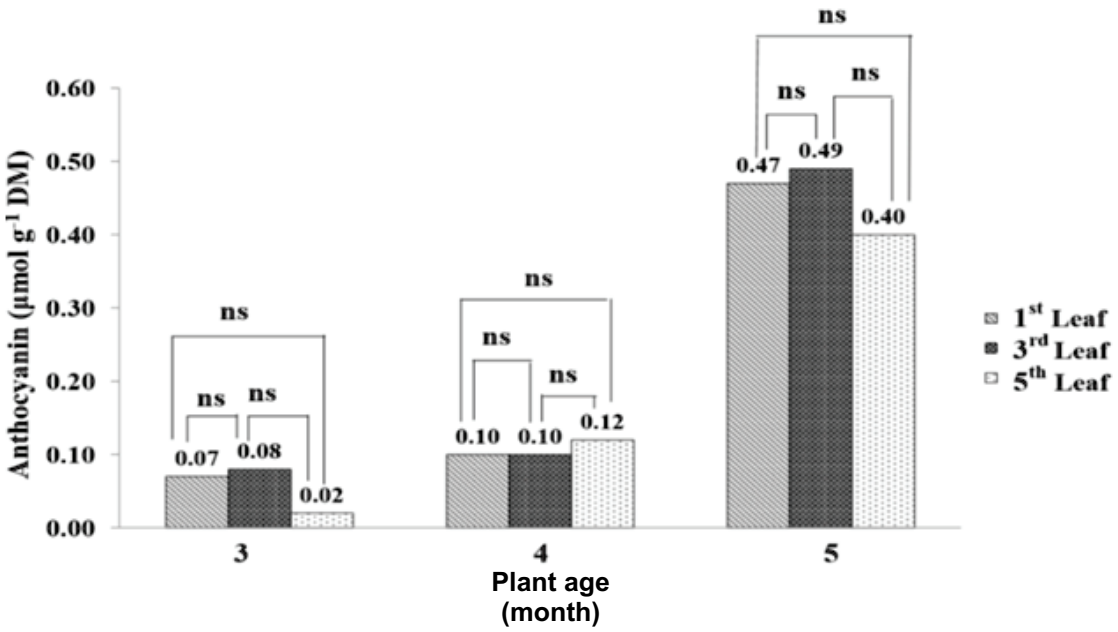

Figure 7. Anthocyanin levels of the 1st, 3rd, or 5th leaf of the three, four and five-month-old torbangun plants.

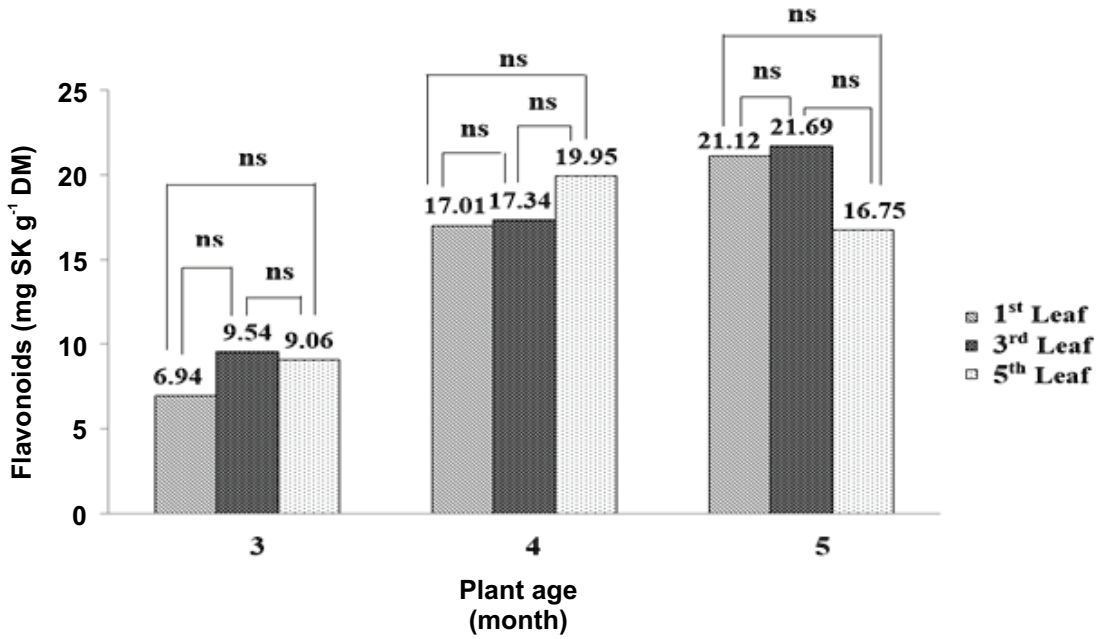

Figure 8. Total flavonoids of the 1st, 3rd, or 5th leaf of the three, four and five-month-old torbangun.

Saponin

The total of saponins concentrations were not affected by the leaves position at three to five-month-old plant (P >0.05, Figure 9). The 1st, 3rd, or 5th leaf position of three to five-month-old plant have increased the total of saponins concentrations.

Correlations of N, P, K and Fe leaf concentrations with shoot dry weight and metabolite production

Leaf positions along the plant and plant ages are important criteria to select leaf samples for leaf nutrient analysis as they correlate with shoot dry weight and metabolite production.

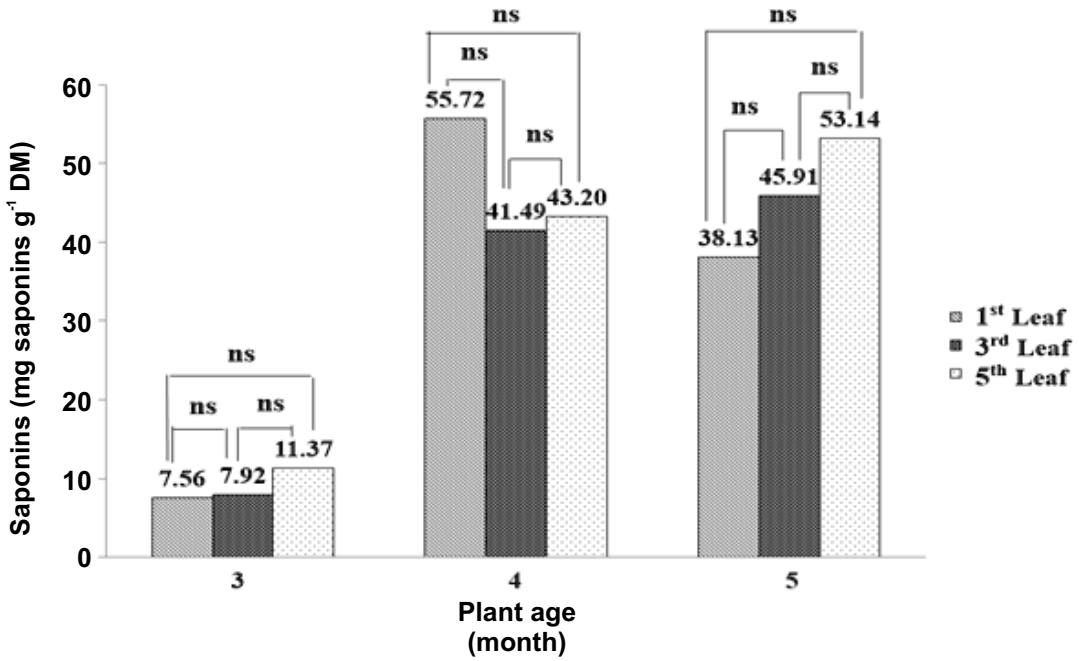

Figure 9. Saponin levels of the 1st, 3rd, or 5th leaf of the three, four and five-month-old torbangun.

Selection of leaf samples for analysis needs to consider to the value of the correlation coefficients (r) between leaf N, P, K, Ca and Fe with shoot dry weight and metabolite production (Table 1). The correlations between leaf nutrients and concentrations of secondary metabolites are presented in Table 2.

K, Ca and Fe levels in the 3rd leaf of five-month-old torbangun plants correlated positively with shoot dry weight, leaf PAL activity and leaf total saponins. Therefore the 3rd leaf can be used as a diagnostic sample for secondary metabolite analysis from torbangun leaves.

Table 1. Correlation (r) between leaf nutrient concentrations with shoot dry weight and metabolites production in the three, four, and five-month-old torbangun plants

Age of plants (Months)	Nutrient	Shoot dry weight	PAL activity	Anthocyanin	Total flavonoids	Total saponins
3	N	0.33	0.10	0.03	0.03	-0.38
	P	-0.29	0.43	0.26	-0.31	-0.21
	K	0.04	0.20	0.56	-0.36	-0.09
	Ca	0.18	-0.65	-0.44	0.44	0.41
	Fe	-0.32	-0.27	-0.20	0.09	0.49
4	N	0.51	-0.53	0.08	-0.26	0.09
	P	0.28	-0.72*	-0.07	-0.34	0.40
	K	0.27	-0.76*	0.36	-0.62	0.63
	Ca	-0.92*	0.37	-0.30	0.35	-0.09
	Fe	-0.26	-0.15	0.34	-0.31	0.36
5	N	0.47	-0.61	0.06	0.64	-0.32
	P	0.48	-0.45	0.21	0.53	-0.42
	K	0.80*	-0.30	0.01	0.55	-0.04
	Ca	-0.34	0.15	-0.25	-0.29	0.46
	Fe	-0.36	0.55	-0.30	-0.39	0.28

Note: * indicates significant correlation; +/- indicates positive or negative correlation.

Table 2. Correlation (r) between the levels of nutrients of the 1st, 3rd, and 5th leaf with shoot dry weight, and metabolites production in the five-month-old torbangun plant

Leaves position	Nutrient	Shoot dry weight	PAL activity	Anthocyanin	Total flavonoids	Total saponins
1	N	-0.81	-0.81	0.42	0.46	-0.68
	P	-0.85	0.22	0.99*	-0.64	0.41
	K	0.97	0.55	-0.71	-0.11	0.38
	Ca	-0.41	-0.17	-0.09	0.84	-0.95
	Fe	-0.99	-0.17	0.93	-0.30	0.02
3	N	0.34	0.69	-0.98	0.09	0.94
	P	-0.85	-0.57	-0.36	-0.96	-0.14
	K	0.99*	0.91	-0.16	0.97	0.62
	Ca	0.91	0.99*	-0.58	0.77	0.90
	Fe	0.63	0.89	-0.88	0.41	0.99*
5	N	-0.67	-0.41	0.99*	0.91	-0.68
	P	-0.39	-0.09	0.94	0.72	-0.88
	K	-0.04	0.26	0.76	0.44	-0.99
	Ca	0.14	-0.16	-0.82	-0.52	0.97
	Fe	0.45	0.70	0.35	-0.05	-0.93

Note: * indicates significant correlation; +/- indicates positive or negative correlation.

The results of this study showed that leaf positions along the plant determined leaf nutrient concentrations. The leaf N, P, and K were the highest in the 1st leaf (Figure 1, 2 and 3) whereas and leaf Ca and Fe were the highest in the 5th leaf (Figure 4 and 5). Liferdi et al. (1995) stated that the change of nutrients in the leaves caused changes in the growth phase. The leaf nutrients decreased in flush phase or generative phase. In this phase leaves undergo translocation of nutrients from old leaves to the younger organs, or to the formation of the fruit, resulting in reduced nutrient concentrations in older leaves. Nitrogen is the most important nutrient for plant growth. Nitrogen is highly mobile so that N deficiency symptoms first appear on older leaves (Jones, 1998). The P content in the plant are normally around 0.15 - 1.00% dry weight, and leaf P of 0.20 – 0.40% are considered to be adequate (Jones, 1998). K levels of the young leaves, petiole and stem decrease with plant age (Jones 1998). Older leaves gradually turn pale and necrotic spots developed at the edge of the leaves (Jones 1998).

Secondary metabolites concentrations (PAL activity, anthocyanin, total flavonoids and total saponins) were not affected by the leaf position (Figure 6, 7, 8 and 9). The 3rd leaf of the five-months-old plant had the highest content of PAL activity, anthocyanin and

total flavonoids. However, this pattern changed when plants were four-month-old. This was possibly related to the differences in rainfall distribution during the collected sample periods. The total rainfall decreased from 843, 527, 419 mm^{-1} months, respectively, when plants were three, four and five-month old. It is possible that this decreased rainfall had increased leaf secondary metabolites concentrations.

K content of the 3rd leaves of the five-month-old plant correlated positively with with shoot dry weight ($r = 0.99$, $P < 0.05$, Table 2). The study by Mardisiswojo and Rajakmangunsudarso (1985) stated that torbangun leaves contain (6.46% of the dry weight of the K_2O) and essential oil (0.043% on a fresh leaf or 0.2% on the dried leaves). K plays roles to clean the blood, prevent infection, reduce pain, creates a sense of calm, and shrink mucous membranes (Mepham, 1987). Sense of calmness generated by torbangun leaves could reduce the pressure caused by the hot weather (Mepham, 1987). Hot weather pressure could reduce appetite, milk secretion, and body weight (Mepham, 1987). Studies by Damanik et al. (2001) showed that torbangun leaves enhanced the secretion of breast milk and overcome women pre menstruation syndromes.

Ca content of the 3rd leaf of the five-month-old plant correlated positively with with PAL activity correlation ($r = 0.99$, $P < 0.05$, Table 2). Phenylalanine ammonia-lyase (PAL) is a catalyser enzyme reaction of phenylalanine as the main precursor in the phenylpropanoid path. PAL will catalyse deamination of L-phenylalanine to trans-cinnamic acid, which is the main intermediate biosynthesis compound of phenolic compounds (Cheng and Breen, 1991; Rivero et al., 2001). There have been limited reports on the correlation of leaf Ca with PAL activity.

Fe content of the 3rd leaf of the five-month-old plant correlated positively with saponins ($r = 0.99$, $P < 0.05$, Table 2). Lactating women who eat torbangun leaves has increased K, Ca, Fe, Zn and Mg in their breast milk, which resulted in the weight increase of the babies significantly (Damanik, 2005). The important role of Fe in the body is to form haemoglobin and helps the body's metabolic processes. The metabolism alter the pro-vitamin A to vitamin A active, oxygen transport, formation of DNA / RNA, synthesis of carnitine to transport fatty acids, collagen synthesis, and synthesis of neurotransmitters (Agus, 2005; Beard et al., 2006). Saponin is the main compound in lactagogue, and sapogenins are glycoside-free portions of the saponins. Sapogenins contain steroid or other triterpene frameworks as their key organic feature (Sunaryadi, 1999), and steroid acts as a hormone that can to increase the production of breast milk (Prajonggo et al., 1983).

Conclusion

This study demonstrated that the 3rd leaves of the five-old-month torbangun plants can be used to determine leaf N, P, K, Ca and Fe content. This study also demonstrated that there were positive correlations between leaf K concentrations with shoot dry weight, between leaf Ca with PAL activity, and between Fe with total saponin of the 3rd leaf of the five-month-old torbangun plants.

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