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Correlations of Nitrogen, Phosphorus, Potassium, Pigments and Total Flavonoids of *Moringa oleifera* Lam. Leaves in the Vegetative and Generative Phases

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Abstract

Moringa oleifera is universally known as the miracle plant or the tree of life. Moringa leaf extract contains phenolic acids and flavonoids, especially flavonols (quercetin, kaempferol, isorhamnetin glycosides) and flavones (apigenin). This study aimed to elucidate any correlations between nutrient, pigment, and flavonoid concentrations for different leaf positions and different growth phases. The results showed that pigment and total flavonoid concentrations increased from the 1st leaf to the 5th leaf, while nutrient concentration decreased. Pigment concentration, total flavonoid, and nutrient concentration were higher in the vegetative phase than the generative phase. The concentration of nutrients in the generative plants displayed a significant positive correlation with chlorophyll concentration. The 1st to 5th leaf of the vegetative plants can be used as indicator leaves for tissue analysis of the moringa plant.

Keywords: bioactive compound, growth phase, indicator leaf, leaf position, nutrient concentration

Introduction

There are about 13 species in the Moringaceae family and the most widely known species was *Moringa oleifera* which is indigenous to south Asia, where it grows in the Himalayan foothills from northeastern Pakistan to northern West Bengal, India (Mahmood et al., 2010). For a variety of purposes, it is now cultivated in the whole tropical and sub-tropical regions of the world (Thapa et al., 2019). Moringa can tolerate a wide range of soil conditions but prefers neutral to slightly acidic soil (pH 6.3 to 7.0). This plant is a fast-growing perennial tree and can reach a maximum height of 7-12 m and a diameter of 20-60 cm. Moringa leaves are bipinnate or more commonly tripinnate, while the flowers are bisexual, fragrant, and surrounded by five unequal and yellow-white petals. The pods are dark green during their development, and contain circa 26 seeds. Moringa plants produce globular seeds, about 1 cm in diameter, with three white papery wings (Roloff et al., 2009; Bashir et al., 2016; Raja et al., 2016).

Moringa is universally known as the miracle plant or the tree of life based on the use as a cure for various diseases and high nutritional content (Oyeyinka and Oyeyinka, 2018; Thapa et al., 2019). These leaves are used to treat several ailments including, swellings, parasitic diseases, cuts typhoid fever, arthritis, malaria, diseases of the skin, genito-urinary ailments, hypertension and diabetes (Konmy et al., 2016). The regular intake of moringa leaves can protect healthy as well as diabetic patients against oxidative damage (Jaiswal et al., 2013) and exhibit antioxidant activity (Fitriana et al., 2016). It display anti-inflammatory activity and antibacterial effects (Vinoth et al., 2012; Ziani et al., 2019), anti-cancer activity (Khalafalla et al., 2010; Jung, 2014; Al-Asmari et al., 2015), can control blood glucose and lipid concentration and prevent hyperglycemia as well as hyperlipidemia (Adisakwattana and Chanathong, 2011). These leaves could be a potential source of compounds with strong antioxidant potential (Verma et al., 2009; Moyo et al., 2012). Moringa leaf extract contains flavonoids and phenolic acids, especially flavonols (myricetin, quercetin, kaempferol, isorhamnetin glycosides) and flavones (apigenin) (Sultana and Anwar, 2008; Coppin et al., 2013; Pakade et al., 2013; Makita et al., 2016; Ziani et al., 2019).

The concentrations of nutrients and bioactive compounds differ in depending on leaf position and

plant growth phase. Tjhia et al. (2018) reported that the concentrations of N, P, and K were found to be higher during the vegetative phase than the generative phase. Respita et al. (2019) reported that the leaves of *Coleus atropurpureus* L. Benth had higher concentrations of chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, anthocyanins, and total flavonoids during the vegetative phase than the generative phase. The highest total flavonoids concentration was obtained from the 4th leaf in both the vegetative and generative phases. Information on the concentrations of nutrients and bioactive compounds at each leaf position can be used to determine indicator leaves for sampling in plant tissue analysis. Information regarding the concentrations of nutrients, pigments, and bioactive compounds, especially flavonoids, in moringa leaves, at various positions and growth phases is still very limited. Therefore, information about the exact leaf position for moringa plant tissue analysis has not yet been obtained. Hence, further research is needed. This study aimed to elucidate any correlations between nutrient, pigment, and flavonoid concentrations at different leaf positions and different growth phases.

Materials and Methods

Plant Materials

Plant material was collected from moringa plants (Figure 1) in Dramaga, Bogor, West Java during vegetative (7-8 months old) and generative (more than 5 years old) growth phases. This experiment was conducted from August until November 2019.

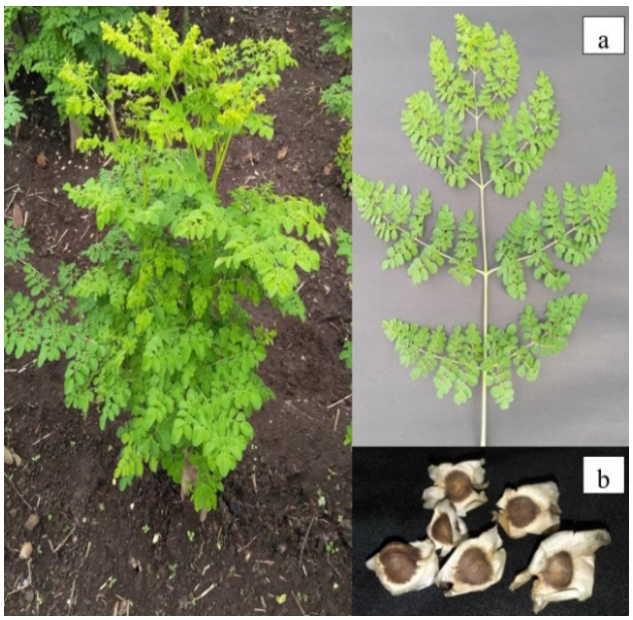

Figure 1. *Moringa oleifera* Lam. plant (left), leaf (a, top right), seeds (b, bottom right)

Leaf samples were collected from 1st, 2nd, 3rd, 4th, and 5th leaves, counted from the shoot tip. Samples were collected from 3 vegetative plants and 3 generative plants as replication. The vegetative plants were not flowering or fruiting and samples were collected on October, while the generative plants were flowering or fruiting and samples were collected August.

Analysis of Leaf Nutrient Concentration

Leaf nitrogen concentration was analyzed using the Kjeldahl method. Phosphorus concentration was measured with a UV-VIS Shimadzu UV-1800 Spectrophotometer and potassium concentration was measured using an PG-990 Atomic Absorption Spectrophotometer. The nutrient analysis was carried out at the Testing Laboratory of the Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University.

Analysis of Pigment Concentration

The concentrations of chlorophyll a, chlorophyll b, carotenoids, and anthocyanins were analyzed using a method that derived from Sims and Gamon (2002). Briefly, 0.0170-0.0240 g fresh leaf was ground and added with 2 ml acetone tris. The extract was centrifuged, 1 mL of supernatant was collected and 3 ml of acetone tris was added. The solution was then vortexed thoroughly. Absorbance was then measured at wavelengths of 470, 537, 647 and 663 nm using a Shimadzu UV-1280 spectrophotometer.

Analysis of Total Flavonoid Concentration

The analysis of the total flavonoid concentration was carried out using the colorimetric aluminum chloride method (Chang et al. 2002). The leaf samples were dried in an oven (60^{o}C) for 48 hours and blended using a blender. The leaf powder was weighed (0.026 g) and placed in microtubes. Subsequently, 1.25 ml of 80% ethanol was added and the mixture stored in a dark container. The quercetin (10 mg) was dissolved in 80% ethanol and diluted to 25, 50 and 100 µg/ml. The diluted solution (0.5 ml) was mixed with leaf extract, 95% ethanol (1.5 ml), 10% aluminum chloride (0.1 ml), potassium acetate 1M (0.1 ml) and distilled water (2.8 ml). This sample solution was incubated at room temperature for 30 minutes, at the end of which, the absorbance was measured at wavelengths of 415 nm using a Shimadzu UV-1280 spectrophotometer. Analysis of pigment and total flavonoid concentrations were carried out in the Postharvest, Biomass, and Spectrophotometry Laboratory of the Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University.

Data Analysis

Nitrogen, phosphorus, and potassium concentrations were correlated with pigment and total flavonoid concentrations by using the Pearson correlation test. The nutrient, pigment, and total flavonoid concentration data were further analyzed *via* a t-student test.

Results and Discussion

Pigment Concentration in the Vegetative and Generative Phases

Chlorophyll and carotenoid concentrations increased from the 1st to the 5th leaf in vegetative plants, except for the 2nd leaf (Figure 2). The 1st, 2nd, 3rd, and 4th leaves displayed chlorophyll a concentration 16.08%, 20.99%, 5.69%, and 3.68% lower respectively than the 5th leaf, while these leaves showed chlorophyll b concentration 17.91%, 25.80%, 7.45%, and 4.15% less than the 5th leaf. Total chlorophyll concentration in the 1st, 2nd, 3rd, and 4th leaves were 16.51%, 22.13%, 6.11%, and 3.79% lower than in the 5th leaf. In contrast, the chlorophyll a, chlorophyll b, and total chlorophyll concentrations in the 3rd and 4th leaves were not significantly different from the 5th leaf. Carotenoids concentration in 1st, 2nd, 3rd, and 4th leaves were 10.09%, 12.76%, 2.42%, and 2.22% lower compared to the 5th leaf, but only the 1st leaf had carotenoids concentration which was significantly different to those found in the 5th leaf. Anthocyanins concentration fluctuated in each leaf positions and the lowest and highest concentrations in the 2nd and 4th leaves. Anthocyanins concentration in 1st, 2nd, 3rd, and 5th leaves were 16.55%, 20.97%, 19.42%, and 13.36% lower than in the 4th leaf. However, the anthocyanins concentration did not significantly differ between leaf positions.

Chlorophyll and carotenoid concentrations increased from the 1st to the 5th leaf in generative plants, except for the 2nd leaf (Figure 3). The 1st, 2nd, 3rd, and 4th leaves showed chlorophyll a concentration 17.04%, 19.09%, 7.93%, and 2.07% lower respectively than those found in the 5th leaf, while these leaves displayed chlorophyll b concentration low of 18.14%, 19.95%, 9.94%, and 1.86% less than the 5th leaf. Total chlorophyll concentration in the 1st, 2nd, 3rd, and 4th leaves were 17.30%, 19.29%, 8.40%, 2.02% lower than in the 5th leaf. Chlorophyll a, chlorophyll b, and total chlorophyll concentrations in the 1st leaf were significantly different from the 5th leaf. Carotenoids concentration in the 1st, 2nd, 3rd, and 4th leaves were 14.76%, 21.95%, 8.88%, and 5.21% lower than in the 5th leaf, but those concentrations did not differ significantly from the 5th leaf. Anthocyanin concentration fluctuated at each leaf position and the lowest and highest concentrations were detected in the 3rd and 4th leaves. Anthocyanin concentrations in the 1st, 2nd, 3rd, and 5th leaves were 3.68%, 9.87%, 18.39%, and 3.18% lower than in the 4th leaf, but those concentrations did not differ significantly from the 4th leaf. Further, the concentration of pigments in the vegetative plants was higher than in the generative plants. The concentrations of chlorophyll a, chlorophyll b, total chlorophyll, anthocyanins, and carotenoids in the vegetative plants were 8.07%, 8.01%, 8.05%, 50.36%, and 12.01% higher than in the generative plants respectively, but only anthocyanins concentration was significantly different between the growth phases.

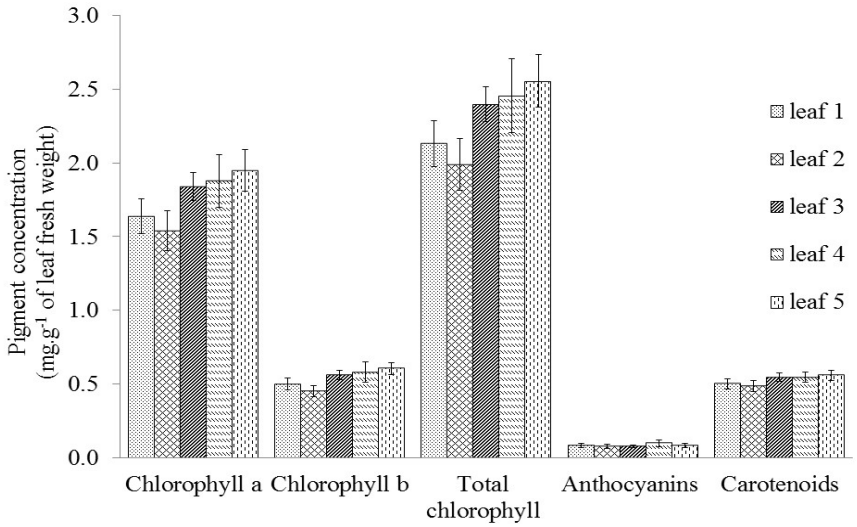

Figure 2. Concentration of pigments (mean ± standard error) in five leaves positions of the vegetative plant

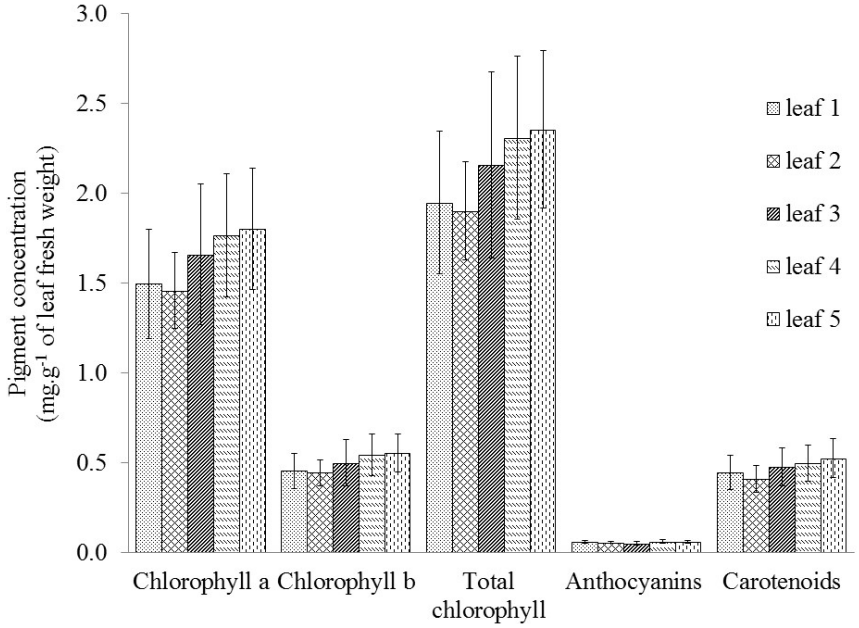

Figure 3. Concentration of pigments (mean ± standard error) in five leaves positions of the generative plant

Nutrient Concentrations in the Vegetative and Generative Phases

Nitrogen, phosphorus, and potassium concentrations decreased from the 1st leaf to the 5th leaf in the vegetative and generative plants (Figure 4). The 2nd, 3rd, 4th, and 5th leaves showed nitrogen concentration 6.56%, 7.73%, 9.55%, and 15.2% lower respectively than in the 1st leaf. Phosphorus concentrations in the 2nd, 3rd, 4th, and 5th leaves were 23.21%, 36.96%, 44.64% and 49.46% lower than in the 1st leaf, whereas the potassium concentrations in the 2nd, 3rd, 4th, and 5th leaves were 5.98%, 18.10%, 21.03%, and 24.21% lower than in the 1st leaf. The 2nd, 3rd, 4th, and 5th leaves did not have significantly different nutrient concentrations compared to the 1st leaf. In the generative plants, nitrogen concentration fluctuated in each leaf position and the highest concentration was found in the 4th leaf, while the lowest was detected in the 2nd and 3rd leaves. Nitrogen concentrations in 1st, 2nd, 3rd, and 5th were 0.86%, 1.08%, 1.08%, and 0.43% lower than those found in the 4th leaf and those concentrations were not significantly different from the 4th. The 2nd, 3rd, 4th, and 5th leaves displayed phosphorus concentrations 10.75%, 23.25%, 23.25%, and 27.5% less than the 1st leaf and only the 2nd leaf had phosphorus concentrations which were not significantly different from the 1st leaf. The 2nd, 3rd, 4th, and 5th leaves had potassium concentrations 4.43%, 13.70%, 18.81%, and 20.06% lower than in the 1st leaf. The concentrations in the 3rd and 4th leaves did not differ significantly from the 1st leaf. Nutrient concentrations were higher in the vegetative plants than the generative plants. Nitrogen, phosphorus, and potassium concentrations in the vegetative plants were higher by 25.12%, 16.57%, and 7.18% respectively compared to the generative plants, and only the potassium concentration was not significantly different between growth phases.

Total Flavonoid Concentration and Total Flavonoid Production in the Vegetative and Generative Phases

Total flavonoid concentration fluctuated at each leaf position in the vegetative plants, while it slowly increased from the 1st to the 5th leaf in the generative plants (Figure 5). The concentration of this compound in the 2nd, 3rd, 4th, and 5th leaves was 2.70%, 4.83%, 0.84%, and 3.65% lower than in the 1st leaf of vegetative plants. Total flavonoid concentration in those four leaves positions was not significantly different from the 1st leaf. In generative plants, the 1st, 2nd, 3rd, and 4th leaves showed total flavonoid concentration of 15.79%, 5.59%, 9.02%, and 2.87% less than the 5th leaf and only the 2nd leaf had significantly difference concentration with the 5th leaf. Total flavonoid concentration in generative plants was 71.22% lower and differ significantly compared to the vegetative plants.

Total flavonoid production increased from the 1st to the 5th leaf in both vegetative and generative plants (Figure 6). The production in the 1st, 2nd, 3rd, and 4th leaves were 43.58%, 34.42%, 15.22%, and 10.27% lower respectively than in the 5th leaf and only production in the 1st leaf differ significantly from the 5th leaf in the vegetative plants. In generative plants, the 1st, 2nd, 3rd, and 4th leaves showed total flavonoid production 38.98%, 20.78%, 11.56%, and 8.55% lower than found in the 5th leaf and all those

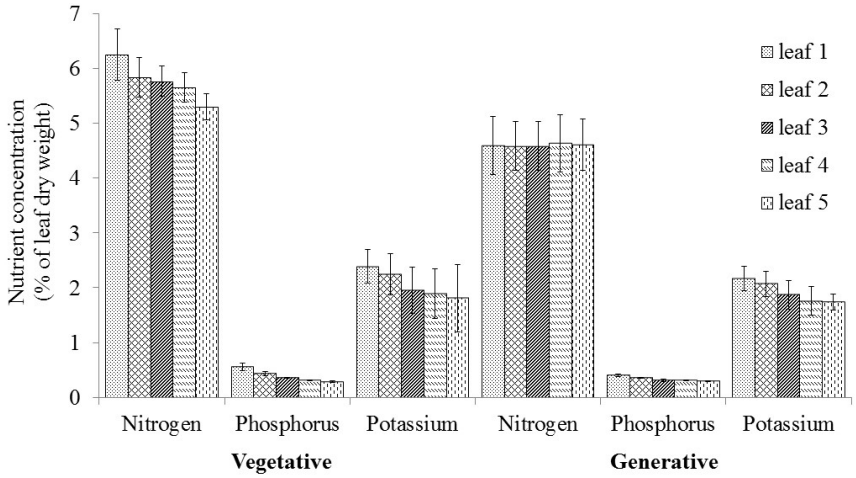

Figure 4. Concentration of nutrients (mean ± standard error) in five leaves positions of the vegetative and generative plants

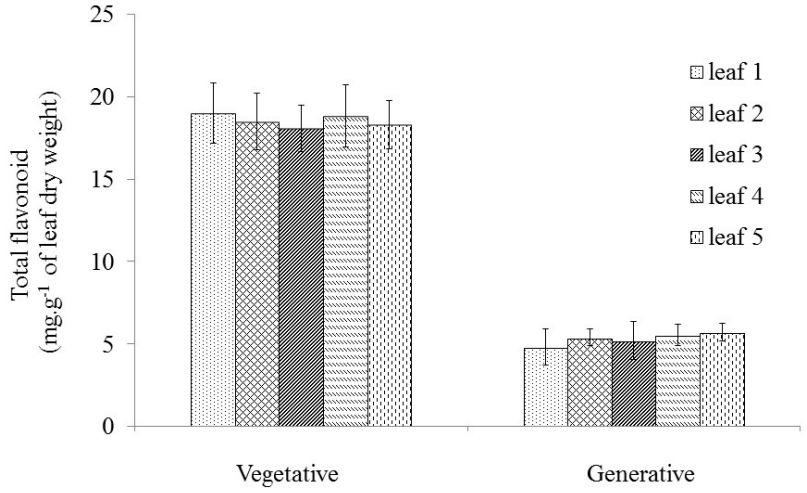

Figure 5. Total flavonoids concentration (mean ± standard error) in five leaves positions of the vegetative and generative plants

productions did not differ significantly from the 5th leaf. Total flavonoid production in the generative plants was 76.30% lower than in the vegetative plants.

Leaf Weights in the Vegetative and Generative Phases

Fresh and dry weights of leaf increased from the 1st to the 5th leaf in vegetative and generative plants (Figure 7). The 1st, 2nd, 3rd, and 4th leaves displayed fresh weight 35.94%, 26.81%, 8.15%, and 7.95% lower than the 5th leaf and dry weight of those leaves were 44.96%, 34.06%, 12.20%, and 10.69% lower respectively than the 5th leaf in the vegetative plants. In generative plants, the 1st, 2nd, 3rd, and 4th had fresh weight 25.55%, 13.80%, 2.76%, and 1.75% lower than 5th leaf and those leaves showed dry weight 26.51%, 12.97%, 4.38%, and 4.76% lower than the the 5th leaf. The generative plants had fresh and dry weights 20.47% and 20.03% lower than the vegetative plants.

Correlations between Nutrient Concentration with Pigment and Total Flavonoid Concentrations in the Vegetative and Generative Phases

Table 1 and Table 2 display the correlations of nutrient concentration with the concentrations of pigments and total flavonoids, and total flavonoid production in the vegetative and generative plants. Significant positive correlations were detected in the 1st and 2nd leaves. Specifically, potassium concentration was correlated with total flavonoid production. In the generative plants, significant positive correlations were found in the 1st, 3rd, 4th, and 5th leaves, specifically concerning nutrient concentration and chlorophyll concentration. Nitrogen concentration was positively correlated with total chlorophyll concentration in the 1st and 5th leaves and with chlorophyll a concentration in the 3rd leaf.

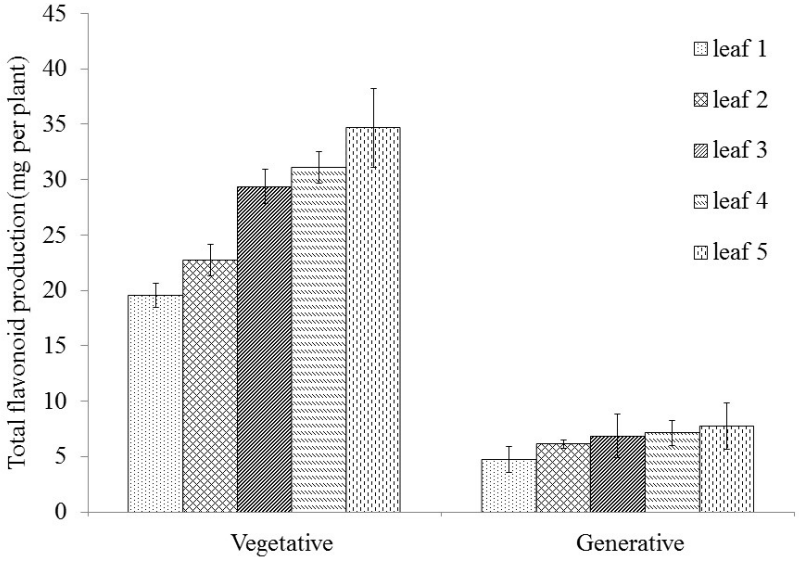

Figure 6. Total flavonoids production (mean ± standard error) in five leaves positions of the vegetative and generative plants

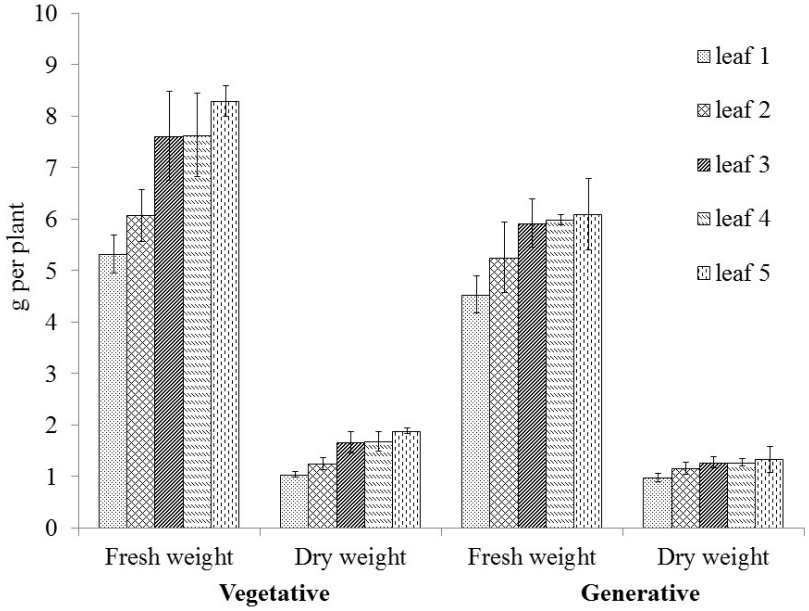

Figure 7. Leaf fresh and dry weight (mean ± standard error) in five positions of the vegetative and generative plants

Furthermore, phosphorus concentration was positively correlated with chlorophyll a concentration in the 1st and 4th leaves and with total chlorophyll concentration in the 3rd leaf. The potassium concentrations in the 1st and 5th leaves positively correlated with chlorophyll b concentration.

and potassium concentrations with pigments and total flavonoid concentrations in five leaf positions of generative plant

Discussion

Generally, moringa leaf pigment concentration increased from the 1st to the 5th leaf, both in the vegetative and generative plants. Sreelatha and Padma (2009) reported that the total carotenoid levels of *Moringa oleifera* leaves were higher in matured leaves than younger leaves. Increased concentrations of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids from the 1st to the 5th leaf were attributed to differences in the development of the upper leaves which were younger than the lower leaves. Li et al. (2016) stated that the leaf position of *Camellia*

Table 1. Correlations between nitrogen, phosphorus, and potassium concentrations with pigments and total flavonoid concentrations in five leaf positions of vegetative plant

Leaf position	Nutrient	Chlorophyll a ($mg.g^{-1}$)	Chlorophyll b ($mg.g^{-1}$)	Total chlorophyll ($mg.g^{-1}$)	Anthocyanin ($mg.g^{-1}$)	Carotenoid ($mg.g^{-1}$)	Total flavonoid ($mg.g^{-1}$)	Total flavonoid production (mg per plant)
1	Nitrogen	0.122	-0.285	0.019	-0.651	0.164	-0.846	-0.999*
	Phosphorus	-0.268	-0.631	-0.367	-0.892	-0.227	-0.576	-0.938
	Potassium	-0.037	0.365	0.066	0.713	-0.080	0.797	0.999*
2	Nitrogen	-0.370	-0.356	-0.367	0.971	-0.544	-0.410	-0.999*
	Phosphorus	-0.515	-0.502	-0.512	0.997	-0.673	-0.258	-0.982
	Potassium	0.377	0.363	0.374	-0.973	0.551	0.403	0.999*
3	Nitrogen	0.298	-0.250	0.174	0.107	0.212	-0.475	0.050
	Phosphorus	-0.972	-0.950	-0.994	-0.999*	-0.989	0.909	-0.999*
	Potassium	-0.379	0.166	-0.258	-0.194	-0.295	0.549	-0.135
4	Nitrogen	-0.205	-0.301	-0.233	0.052	-0.262	-0.644	0.921
	Phosphorus	-0.774	-0.707	-0.756	-0.910	-0.736	0.976	-0.080
	Potassium	0.364	0.454	0.389	0.113	0.417	0.509	-0.973
5	Nitrogen	0.074	-0.223	0.007	-0.326	-0.045	-0.121	-0.300
	Phosphorus	-0.991	-0.906	-0.979	-0.856	-0.967	0.996	0.996
	Potassium	-0.388	-0.099	-0.326	0.007	-0.276	0.431	0.588

Note: *indicates significant correlation according to Pearson correlation test at α = 0.05; +/- indicates positive or negative correlation

Table 2. Correlations between nitrogen, phosphorus, and potassium concentrations with pigments and total flavonoid concentrations in five leaf positions of generative plant

Leaf position	Nutrient	Chlorophyll a ($mg.g^{-1}$)	Chlorophyll b ($mg.g^{-1}$)	Total chlorophyll ($mg.g^{-1}$)	Anthocyanin ($mg.g^{-1}$)	Carotenoid ($mg.g^{-1}$)	Total flavonoid ($mg.g^{-1}$)	Total flavonoid production (mg per plant)
1	Nitrogen	0.988	0.979	0.998*	0.217	0.812	-0.113	-0.517
	Phosphorus	0.999*	0.934	0.996	0.060	0.894	0.046	-0.375
	Potassium	0.940	0.999*	0.966	0.400	0.686	-0.301	-0.671
2	Nitrogen	0.928	0.997	0.965	-0.081	0.594	-0.201	-0.981
	Phosphorus	0.768	0.973	0.836	0.231	0.317	-0.494	-0.872
	Potassium	0.931	0.996	0.967	-0.088	0.600	-0.194	-0.982
3	Nitrogen	0.999*	0.956	0.996	0.264	0.931	-0.121	0.065
	Phosphorus	0.990	0.991	0.997*	0.417	0.860	-0.281	-0.098
	Potassium	0.838	0.955	0.873	0.761	0.564	-0.660	-0.509
4	Nitrogen	0.980	0.993	0.993	-0.304	0.804	0.354	0.618
	Phosphorus	0.999*	0.941	0.994	-0.511	0.918	0.556	0.780
	Potassium	0.711	0.896	0.765	0.265	0.350	-0.213	0.090
5	Nitrogen	0.991	0.988	0.998*	-0.234	0.944	0.014	0.655
	Phosphorus	0.949	0.818	0.925	-0.638	0.993	0.453	0.921
	Potassium	0.960	0.999*	0.977	-0.087	0.885	-0.135	0.536

Note: *indicates significant correlation according to Pearson correlation test at α = 0.05; +/- indicates positive or negative correlation

sinensis represented a specific developmental stage that influenced both photosynthesis and respiration. Concentrations of chlorophyll a, chlorophyll b, and total chlorophyll increased gradually with leaf maturity, while respiration rate and soluble sugar decreased, and starch concentration increased with leaf maturity. This indicates that the ordinary source-sink relationship was altered during tea leaf development. Flexas et al. (2012) detected modifications of the structure and physiological characteristics in developing leaves. Cell size, tissue composition, and leaf thickness were determined in this phase. The synthesis of chlorophyll and photosynthetic enzymes occurred simultaneously with a multiplication of chloroplasts, an increase in leaf area, and the formation of leaf internal structures.

Leaf area influences pigment concentration and dry weight. Xie and Luo (2003) showed that the leaf area determined light interception and influenced dry matter production. The below leaves were wider than the upper leaves which can be seen from the higher dry weight of these leaves. The results of the study of Hgaza et al. (2009) showed there was an increase in leaf area, stomatal conductance, and photosynthetic capacity of *Dioscorea alata* L. from the apex to base leaves (from 1st to 4th leaf).

The pigment concentration was higher in the vegetative plant than the generative plants. The low concentration of pigments in the generative plants was likely due to sampling occuring during the dry season, while the leaf samples of the vegetative plants were taken at the beginning of the rainy season. Previously, Nouman et al. (2013) showed that antioxidants, total phenolics, photosynthetic pigments, P, K, Ca, Mg, and crude protein contents of moringa leaves were higher in the hot rainy season than the hot dry season. Mabapa et al. (2018) reported that the photosynthetic rate, stomatal conductance, and transpiration rate of *Moringa oleifera* leaves were decreased during the summer season, while the photosynthetic rate and stomatal conductance especially, were higher during the winter season when temperatures were low. The low concentration of pigments in the generative plants was caused by a source-sink relationship. The sample of the generative plants in this study was from flowering and fruiting plants. Flower and fruit were strong sinks so that nutrients and photosynthates in the leaves (source) were allocated mostly to the formation of reproductive organs and not to leaf growth. Taiz and Zeiger (2002) stated that the tip of the canopy and root were the main sinks during the vegetative phase while the seeds and fruits become the dominant sink during the reproductive phase. This aligns with a study by Berezina et al. (2017), which showed a low anthocyanin content in American cranberry leaves (*Vaccinium macrocarpon* Ait.) during the flowering phase. This was because anthocyanins have photoprotective and antioxidant functions, but the leaf photosynthetic apparatus terminated its formation and acquired resistance to photodestruction in that phase.

The total flavonoid concentration in the vegetative plants fluctuated across leaf positions. However, the total flavonoid concentration slowly increased from the 1st leaf to the 5th leaf in the generative plants. This was because younger leaves were sinks and received nutrients and photosynthates supplied by older leaves and these resources were used for leaf growth and not the synthesis of flavonoids. Jahan et al. (2015) found that the total flavonoid content of moringa leaves was higher in matured leaves than in tender leaves because the analyzed mature leaves possessed a higher chlorophyll content and leaf area. A further study by Monsurat and Catherine (2016) on moringa plants showed that mature leaves had a higher phytochemical quantity compared to immature leaves. Sreelatha and Padma (2009) reported a higher total phenolics and flavonoids content in matured leaves than in the tender leaves of moringa. Furthermore, the study of Nisa et al. (2019) showed mature leaves of *Carica papaya* L. had higher antioxidant activity and total flavonoid concentration than young leaves. The total flavonoid content was higher in mature leaves than young leaves and dramatically increased in old leaves of *Clausena lansium* (Lour.) Skeels plant (Chang et al., 2018).

Total flavonoid concentration in the vegetative plants was much higher than the generative plants. The study of Berezina et al. (2017) on American cranberry plants (*Vaccinium macrocarpon* Ait.) showed that phenolic content decreased during plant development phases. Physiological changes in vegetative plants that developed into reproductive plants were followed by a decrease in the accumulation of secondary metabolites. The flavonoid content in American cranberry leaves decreased during the flowering phase due to flavonoids being used up by plants during pollination and sexual reproduction processes. Mutalib (2015) reported the leaves of *Plantago major* had a higher total phenolic content during the vegetative period than the generative period, while Jimoh et al. (2019) showed increased antioxidant activity, as well as total phenolic, and flavonoid content in the leaf and herbaceous stem of *Amaranthus caudatus* L. during the pre-flowering stage than in the flowering and post-flowering stages. Flavonoid production increased with increasing leaf position at each growth phase. This effect can be attributed to the dry weight increased from the 1st to the 5th leaf. Aziz (2015) stated that the production of bioactive compounds was the result of the

multiplication of biomass with the concentration of these bioactive compounds and this study production of compounds was calculated on this basis. Flavonoid production was found to be higher in the vegetative plants than the generative plants. Besides differences in leaf weight, this may also be explained by the source-sink relationship. The high leaf weight in the vegetative plants indicated that nutrients and photosynthates were allocated preferentially to leaf growth, while nutrients and photosynthates were allocated largely to the growth and development of reproductive organs in the generative plants so that the leaf weight in these was lower.

Nutrient concentration decreased with increasing leaf position in the vegetative and generative plants. This can be explained by nitrogen, phosphorus, and potassium being mobile within the plant. The results of Agamou et al. (2015) in moringa plants showed that mature leaves contained higher protein, fiber, divalent cations (Ca, Mg, Fe, Mn, Cu) and bioactive compounds, whereas young leaves contained more carbohydrates, total carotenoids, potassium, phosphorus, and zinc. The concentrations of potassium, phosphorus, and zinc were lower in the generative plant due to stronger sinks, namely flowers, fruits, and seeds so that nutrients and photosynthates were more allocated to the growth and development of the reproductive organs. Marschner (2012) stated nitrogen, phosphorus, and potassium highly mobile in phloem and can be remobilized from mature leaves to areas of new growth. Remobilization of nutrients becomes important during reproductive growth and formation of seeds, fruits, and storage organs. Root activity and nutrient uptake generally decline in this growth phase due toa decreased supply of carbohydrates to the root which is caused by the increased competition between sinks so that the concentration of nutrients in vegetative organs decreases dramatically during the reproductive phase.

Nutrients concentrations in five leaves positions had no significant correlation with anthocyanins, carotenoids, and total flavonoid concentrations in both growth phases. Besides, nutrient, pigment, and total flavonoid concentrations, as well as total flavonoid production were higher during the vegetative phase compared to the generative phase. The results of this study demonstrated that the leaves at the 1st to 5th position during the vegetative phase can be used as indicator leaf for sampling in measurement of tissue analysis of moringa plant.

Conclusion

Pigment and total flavonoid concentrations increased from the 1st leaf to the 5th leaf, while nutrient concentration decreased. Importantly, pigment, total flavonoid, and nutrient concentrations were higher in the vegetative plants than the generative plants. Nitrogen, phosphorus, and potassium concentrations did not correlate positively with pigment and total flavonoid concentrations in the vegetative plants, whereas nutrient concentrations were significantly and positively correlated with chlorophyll concentration in the generative plants.

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