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Effects of leaf ages, altitude and clone types on nutrient elements and antioxidant activity of tea (*Camellia sinensis* L. (O) Kuntze) in tropical conditions

Wisnu Eko Murdiono [a,g], Nur Amirah Syafiqah Salman [a], Nor Asma Ab Razak [c], Mohd. Izuan Effendy Halmi [d], Jean Wan Hong Yong [e,*], Abbe Maleyki Mhd. Jalil [f], David F.R.P. Burslem [h], Khairil Mahmud [a,b,*]

[a] Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[b] Biodiversity Unit, Institute of Bioscience (IBS), Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[c] Natural Medicine and Product Research Laboratory, Institute of Bioscience (IBS), Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[d] Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[e] Department of Biosystems and Technology, Swedish University of Agricultural Sciences, Alnarp, Sweden
[f] School of Nutrition & Dietetics, Faculty of Health Sciences, Universiti Sultan Zainal Abidin (UniSZA), Terengganu 21300, Malaysia
[g] Agronomy Department, Faculty of Agriculture, Universitas Brawijaya, Malang, Indonesia
[h] School of Biological Sciences, University of Aberdeen, Aberdeen, AB24 3UU Scotland, United Kingdom

A R T I C L E I N F O

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A B S T R A C T

Tea is a globally popular heritage beverage consumed by over three billion people. The unique taste and health benefits of tea are linked to its nutrient composition and antioxidant activity (AOA). As a plant species, tea's nutrient elements and AOA vary based on season, altitude, clone type and leaf age. This study examined the nutrient composition and AOA of young and mature tea leaves from four clones (BC1248, TRI2024, AT53 and TV9) grown at different altitudes under tropical conditions in Malaysia. The results demonstrated that altitude and clone type significantly influenced ($p < 0.05$) foliar nutrient elements and AOA. Young tea leaves have higher nutrient levels and AOA than mature leaves across all clones. Interestingly, the foliar nutrient availability was higher in the highlands, although the variation across the four clones was insignificant ($p > 0.05$). On the other hand, foliar nutrient elements varied significantly among lowland tea clones, except for N and Ca. The highest AOA was recorded in young tea leaves of clone BC1248 at the lowland plantations, with total polyphenol contents (TPC), 2,2-diphenyl-1-picrylhydrazylradical (DPPH IC_{50}), and ferric reducing antioxidant power (FRAP) values of 19.60 ± 0.15 mg GAE/g, 50.70 ± 1.86 μg/mL, 2.10 ± 0.14 mM Fe (II)/g, respectively. The DPPH IC_{50} and FRAP varied significantly ($p < 0.05$), except for TPC among the lowland and highland clones. Based on principal component analysis (PCA), we identified that the tropical lowlands of Malaysia were more suitable for growing tea with high AOA. These findings provide valuable insights for growers to develop sustainable tea farming strategies, ensuring optimal yield and targeted quality under tropical conditions.

1. Introduction

Tea (*Camellia sinensis* L. (O) Kuntze) was exclusively consumed by the Chinese as a beverage around 2000 years ago. Nowadays, consuming more than three billion cups of tea daily reflects its global popularity (Pan et al., 2022; Zhao et al., 2022). Tea is known to have beneficial impacts on health and these advantages correspond to a substantial quantity of catechins, a major component of tea's polyphenols, which have been described as powerful antioxidants by numerous means, such as neutralising free radicals, stabilising transition metal ions such as iron and copper, and hindering oxidative enzymes (Tian et al., 2022). In addition to polyphenols, tea leaves contain amino acids, caffeine, vitamins, minerals and trace elements (Podwika et al., 2018). Certain minerals and trace elements in tea leaves play an important role in human metabolism. Some of the nutrient elements such as Fe, Cu, Mn and Zn are essential for basic processes in the human body (Podwika

* Corresponding authors.
E-mail addresses: jean.yong@slu.se (J.W.H. Yong), khairilmahmud@upm.edu.my (K. Mahmud).

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et al., 2018). Therefore, both the nutrient composition and the polyphenolic compounds are beneficial for health.

Several researchers have reported that both nutrient elements and antioxidant activity (AOA) in tea vary according to the season (Govindasamy et al., 2023), altitude (Guo et al., 2025; Martono et al., 2016; Xiang et al., 2021), leaf age (Chan et al., 2007; Izzreen et al., 2013) and clone type (Amirah et al., 2023b; Zhang et al., 2020). Govindasamy et al. (2023) reported that the summer-grown tea had the highest antioxidant activity across all cultivars evaluated, followed by those grown during the pre-monsoon, winter and monsoon periods. Altitude plays a greater role as it affects both nutrient elements and the AOA of tea. The altitudinal differences caused significant temperature differences, altering growth rates and patterns. This was observed throughout tea cultivation across >50 countries worldwide (Xia et al., 2020), leading to tea nutritional elements and AOA variation. Tea from higher altitudes may have different concentrations of minerals like Ca and Mg, which are influenced by the soil and climatic conditions (Lin et al., 2015). Meanwhile, the AOA of tea measured through ABTS and DPPH assay tends to decrease with increasing altitude (Chakraborty et al., 2015).

The leaf age considerably affects tea's chemical composition, particularly its phenolic content, which consists of flavanols, flavonols, flavones, proanthocyanins, and phenolic acids. They are estimated to make up about 20–35 % of the tea foliar dry weight, with flavan-3-ols (also known as catechins) accounting for 60–80 %. Earlier studies have shown significant tea leaves' phenolic makeup alters during maturation. Paiva et al. (2021) reported that young tea leaves contained higher total catechin content compared to mature tea leaves. Liu et al. (2020b) confirmed that there were significant changes in the phenolic profiles during tea leaf aging. As tea leaves matured, there was a general and concomitant drop in flavanol and phenolic acid content.

Tea clone types demonstrate significant variations in nutrient concentrations and AOA. Amirah et al. (2023b) reported significant variations in nutrient concentration and AOA among seven tropical lowland clones evaluated in Malaysia. Similarly, clone Iran 100 also had the highest total phenolic and flavonoid content among 12 high-yielding tea clones in Iran (Gonbad et al., 2015). Clone 6/8 produced high-quality green tea with high polyphenols but less caffeine content among five clones evaluated in Ethiopia, particularly when treated with high (300 kg ha^{-1}) nitrogen levels (Benti et al., 2022). Moreover, this clone produced an excellent full aroma, balanced bitterness and a slightly sweeter cup in all nitrogen treatments than other clones.

Tea has been planted in Malaysia in two distinct altitudes for nearly a century. Previous tea research in Malaysia was conducted by Chan et al. (2007), Izzreen et al. (2013), and Amirah et al. (2023b), primarily focused on lowland plantations. Chan et al. (2007) and Izzreen et al. (2013) investigated the effects of leaf maturity tea AOA, while Amirah et al. (2023b) examined both nutrient elements and AOA across distinct tea clones. However, the influence of altitude differences (lowland versus highland) on nutrient elements and the AOA between tropical tea

Fig. 1. The location map of tea clones (BC1248, TRI2024, AT53, TV9) cultivated in a) lowland and b) highland plantations of Malaysia.

clones remains inadequately explored. Understanding the influence of tea clones, leaf ages, and altitudes is crucial for optimizing sustainable tea cultivation and enhancing the quality of tea-related products. Therefore, this study investigated the nutrient elements and AOA of young and mature leaves from four distinct tea clones (AT53, BC1248, TRI2024 and TV9) cultivated at lowland and highland plantations under tropical conditions in Peninsular Malaysia.

2. Materials and methods

2.1. Study sites

The tea collection site was selected from tropical lowland and highland plantations in Malaysia (Fig. 1). The Cameron Highland is located at Pahang, approximately 1400 m above sea levels (a.s.l), which has an average temperature ranging from 18 – 25 °C with an average humidity of 79 – 92 % and 152.7 – 1077.8 mm rainfall. Meanwhile, Bukit Cheeding Plantation is located in Banting, Selangor, approximately 20 m a.s.l, having temperatures ranging from 28 – 31 °C, humidity (74 – 86 %) and rainfall (53.6 – 596.3 mm). There were four clones planted in both plantations, namely: AT53, TV9, BC1248 and TRI2024. The tea plants sampled in this study were approximately 5 years old at the time of sampling, suggesting they were planted around 2017.

Based on the information provided by the officials at the BOH plantation, fertilization management for both tea plantations follows a carefully planned triennial application schedule (three times annually). The fertilization program incorporated a balanced nutrient approach, utilizing a blend of essential macronutrients, including N, P, K and Mg. The total amount of fertilizer applied is 1 ton ha^{-1} $year^{-1}$ for both plantations. This systematic fertilization approach ensures optimal nutrient distribution and supports the sustained nutritional requirements of tea crops throughout their growth cycle. Sample collection was conducted approximately two months after fertilization was scheduled.

2.2. Plant materials

Four tea clones utilized in this study are TV9, TRI2024, AT53 and BC1248. All four clones were assamica varieties. The tea clone TV9 is one of India's widely cultivated tea clones. The origin and development of TV9 can be traced back to the extensive tea breeding programs conducted by the Tea Research Association (TRA) at the Tocklai Experimental Station (TES) in Assam, India. This program focused on collecting and preserving germplasm based on specific phenotypic traits. The potential clone generated from this program is given a "TV" prefix, which stands for "Tocklai Vegetative". Two-thirds of the tea growing area of North East India is covered by Tocklai vegetative (TV) cultivars (Deka et al., 2021). The program generated numerous tea clones, including TV9. TV9 is a high-yielding clone that tolerates waterlogged conditions better than many other clones, making it ideal for flood-prone areas and offering good resistance to certain fungal diseases (Lloyd et al., 2025). TV9 is also noted for its high content of tannins, proanthocyanidins, and antioxidants. These properties make it a valuable clone for producing high-quality tea with beneficial properties (Das et al., 2024).

Tea clone TRI2024, or some simply call it 2024, originated in Sri Lanka and was developed by the Tea Research Institute (TRI). It is a part of the 2000 series of clones released by the TRI, derived from a single plant designated as ASM 4/10 (Anandappa, 1992). The 2000 series clones, including TRI2023, TRI2024, TRI2025, TRI2026 and TRI2027, were planted in approximately 55 % of Sri Lanka. This was due to the wide adaptability of the 2000 series clone, which can be planted across distinct altitudes. The clone TRI2024 has a high-yielding and rooting performance and a high tolerance for nematodes *Radopholus similis* (Gunasekare, 2012).

The AT53 clone is a distinct clone cultivated at BOH's lowland tea estate in Bukit Cheeding, Selangor. This particular clone is used to produce "Bukit Cheeding No 53″, a popular black tea label from BOH Plantations. According to a study by Chan et al. (2007), this black tea variety is noted for its exceptional sensory qualities, especially its bold, full-bodied character and rich, invigorating aroma. This is likely attributed to the significant concentrations of foliar nutrients, such as K and Mg, as reported by Amirah et al. (2023b).

Similar to AT53, clone BC1248 is a specific clone cultivated at Bukit Cheeding, a lowland tea estate of BOH Plantation. Internally, BOH refers to this clone using the prefix "BC", identifying it as BC1248. It is a designation that confirms its development and cultivation specifically for the Bukit Cheeding estate. Although detailed information about its origin remains undisclosed, it is known that BOH has selectively developed unique clones tailored to thrive in the local environmental conditions. A recent study by Amirah et al. (2023b) reported that among seven clones evaluated in lowland tea plantations, clone BC1248 is a potential clone with high AOA. The lowest DPPH IC_{50} and high FRAP assay values supported this.

2.3. Samples collection

A total of four tea clones, namely AT53, TV9, BC1248 and TRI2024 (Fig. 2) were sampled between October 2021 – March 2022 from lowland and highland tea plantations, respectively. Leaf sampling sites were randomly selected (Table 1), triplicated for young (bud and the first two leaves) and mature leaves (4th to 7th leaves) respectively (Fig. 3). Tea leaves were put in a paper bag during transportation to the laboratory. Fresh tea leaves were washed and rinsed several times using water to remove any remaining materials before oven-dried at 60 °C for four days. The samples then were ground to get the fine powdered texture for further foliar nutrient elements and antioxidant analysis.

2.4. Foliar nutrient element analysis

Total nitrogen (N) was analyzed using a CNS analyzer (TruMac Series Marco Determinator, Leco Corporation, USA; software used for analysis was CNSTruMax Determinator version 1.1x,); while other foliar nutrient elements (P, K, Ca, Mg, Al, and Fe) were analyzed by ICP–OES (Optima 8300, ICP–OES, PerkinElmer, USA). Prior to ICP–OES analysis, tea leaves were digested using the dry-ashing method following Alarefee et al. (2021).

2.5. Antioxidant activity (AOA) analysis

Tea leaves were extracted using 80 % aqueous methanol by digital ultrasonic bath following Bakht et al. (2019) with minor modifications by using 40 °C for 30 min before AOA analysis. The extract produced was evaluated for its TPC, DPPH and FRAP assay according to Amirah et al. (2023b). The TPC was evaluated employing the Folin-Ciocalteu solution with gallic acid, which served as a reference. Ten-fold diluted tea extract (15 μL) was combined with distilled water (240 μL) and 0.25 N Folin-Ciocalteu reagent (15 μL), and then mixed thoroughly. After 3 min of dark incubation at room temperature, 30 μL of 1 N Na_2CO_3 was added. Following 2 h of dark incubation at room temperature, absorbance was measured at 765 nm. In the DPPH assay, 20 μL of tea extract was added to 180 μL of DPPH solution (150 μmol L^{-1}) and incubated for 40 min in the dark. Absorbance was measured at 517 nm, with 80 % methanol used as a blank. L-ascorbic acid served as the positive control. The antioxidant activity was expressed as an IC_{50} value, calculated using GraphPad Prism 8 software (GraphPad Software, San Diego, CA, USA). In the FRAP assay, the FRAP reagent needs to be freshly prepared. The reagent was made by mixing acetate buffer (0.3 M, pH 3.6), 10 mM TPTZ in 40 mM HCl and 20 mM FeCl3 in a 10:1:1 ratio, then warmed at 37 °C. A mixture of FRAP reagent (280 μL) and tea extract (20 μL) was incubated at 37 °C for 30 min and measured the absorbance at 593 nm.

Fig. 2. The shoots of the four selected tea clones: a) BC1248, b) TRI2024, c) AT53, d) TV9, cultivated in lowland and highland plantations of Malaysia.

Table 1
Geographical locations of lowland and highland tea clones in Malaysia.

Tea Clones	Lowland (20 m a.s.l)		Highland (1400 m a.s.l)	
	North latitude (°)	East longitude (°)	North latitude (°)	East longitude (°)
AT53	2.92450	101.57789	4.52340	101.39976
TV9	2.91870	101.57658	4.52110	101.40415
BC1248	2.92172	101.58327	4.52091	101.40018
TRI2024	2.92813	101.58134	4.52322	101.39959

2.6. Statistical analysis

All analytical results were based on the average of three replicates and were analyzed using RStudio version 4.1 (Team R Development Core, 2021). To assess the variations in nutrient elements and antioxidant activity (AOA) among different tea clones, One-way ANOVA was performed, followed by Tukey's HSD test for pairwise comparisons. Two-way ANOVA was used to explore the potential interactions between clone type and altitude on the nutrient composition and AOA of tea leaves. In addition, PCA (principal component analysis) was conducted to examine the relationship between foliar nutrient elements and AOA A significance level of 95 % confidence ($p < 0.05$) was used for all

Fig. 3. The definition of young and mature tea leaves for this experiment.

statistical tests.

3. Results

3.1. Foliar nutrient elements

The foliar nutrient concentration of the young and mature tea leaves across four clones at the lowland and highland was shown in Table 2. Foliar nutrient concentrations of P, K, Mg, Fe and Al were significantly varied among young tea leaves' lowland clones. Foliar P was the only element that varied significantly among mature tea leaves. Among the four clones grown in the lowland plantation, TV9 and BC1248 had the highest foliar P concentration for young (11.80 mg g^{-1}) and mature (9.50 mg g^{-1}) tea leaves, respectively. Meanwhile, clone AT53 had the highest foliar K (1.84 mg g^{-1}), Mg (0.80 mg g^{-1}), Fe (13.00 mg g^{-1}) and Al (16.60 mg g^{-1}).

In the highland tea plantation, we discovered that foliar nutrient concentrations of young tea leaves showed insignificant variation ($p > 0.05$) among the four tea clones evaluated. On the other hand, only foliar K concentration varied significantly among mature tea leaves, with Clone AT53 having the highest concentration (8.75 mg g-1). Based on foliar nutrient concentration, we considered clone AT53 as a premium clone in both plantations since it had the highest foliar Mg, Fe and Al concentration at the lowland plantation as well as foliar K concentration regardless of elevations.

A two-way ANOVA was utilized to investigate the role of clones and altitude on foliar nutrient concentrations (Table 4) of distinct tea leaf ages. In young tea leaves, the clone type effect was only significant ($p < 0.05$) on foliar Mg, Fe, and Al, while altitude significantly influenced all the nutrient elements ($p < 0.05$). The interaction between the clone and altitude was mostly insignificant, except for Fe and Al ($p < 0.05$). In mature tea leaves, clone type significantly affected foliar P and K ($p < 0.05$), while altitude significantly influenced all the nutrient elements ($p < 0.05$). The interaction between clone and altitude was insignificant for all foliar nutrient ($p > 0.05$).

3.2. Antioxidant activity (AOA) of tea

Antioxidant activity (AOA) assay using TPC, DPPH and FRAP of young and mature tea leaves from both plantations was presented in Table 3. DPPH was expressed as IC_{50}, and samples with smaller IC_{50} values generally possessed better antioxidant performance. DPPH IC_{50} values varied significantly among four clones for young and mature leaves from lowland plantations, with clone BC1248 having the best antioxidant performance. In contrast, TPC and FRAP displayed insignificant variation ($p > 0.05$) among four clones for both young and mature leaves derived from lowland plantations.

In the highland plantation, we found that mature leaves' DPPH and FRAP of young tea leaves varied significantly among the four clones examined. On the other hand, young leaves' DPPH, FRAP of mature leaves and TPC from both leaves in highland plantation showed no significant variation among the population. Clone TRI2024 (127.00 μg/mL) and BC1248 (1.49 mM Fe (II)/g) had the best antioxidant performance for DPPH IC_{50} and FRAP, respectively.

A two-way ANOVA was utilized to investigate the role of clones and altitude on AOA (Table 5) of distinct tea leaf ages. In young tea leaves, the clone-type effect was significant ($p < 0.05$) on DPPH and FRAP, while altitude significantly influenced all the AOA parameters evaluated ($p < 0.05$). The interaction between the clone and altitude was only significant on DPPH ($p < 0.05$). In mature tea leaves, clone type significantly affected DPPH and FRAP ($p < 0.05$), while altitude only significantly influenced FRAP ($p < 0.05$). The interaction between clone and altitude was only significant for DPPH ($p < 0.05$).

3.3. Relationship between nutrient concentration and AOA of tea

The population mean values of seven nutrient elements and three antioxidative assays for young tea leaves grown in both plantations displayed the first axis explaining 81.7 % of the variation in the data and a second axis explaining 5.42 % of the variation (Fig. 4a). The first axis shown a positive correlation with all variables (loadings 0.71 – 0.91) (Table 6). The equivalent PCA for mature tea leaves grown in different plantations is displayed by the first axis, which explains 68.6 % of the variation (Fig. 4b). This axis shows a positive correlation with all the seven foliar nutrient elements (loadings 0.69 – 0.89) and FRAP content (loadings 0.82). The Second PC axis, which explained 18.95 % variations, captured variations in tea's TPC and DPPH content (loadings 0.70 and 0.88) (Table 6).

Table 2
Foliar nutrient concentration of the young and mature leaves tea clones at lowland and highland of Malaysia.

Altitude	Leaf ages	Clone	N %	P mg/g	K	Ca	Mg	Fe	Al
Lowland	Young leaf	BC1248	4.22±0.40	11.10±0.20^{ab}	0.26±0.06^{b}	0.61±0.11	0.22±0.07^{b}	**12.80 ± 0.47^{a}**	4.53 ± 0.69^{b}
		TRI2024	3.79±0.20	9.45±0.30^{b}	0.31±0.12^{b}	0.38±0.04	0.14±0.06^{b}	6.01 ± 0.68^{b}	**16.20 ± 0.12^{a}**
		AT53	3.55±0.20	10.20±0.60^{ab}	**1.84±0.38^{a}**	0.70±0.08	**0.80±0.16^{a}**	**13.00 ± 0.79^{a}**	**16.60 ± 0.78^{a}**
		TV9	4.00±0.30	**11.80±0.50^{a}**	0.15±0.04^{b}	0.68±0.09	0.11±0.03^{b}	**10.30 ± 0.37^{a}**	3.66 ± 0.42^{b}
		Mean	3.88±0.10	10.66±0.30	0.64±0.23	0.60±0.06	0.32±0.09	8.67±1.12	10.67±2.02
		p-value	0.423	**0.019***	**0.001****	0.083	**0.002****	**0.000*****	**0.000*****
	Mature leaf	BC1248	3.68±0.40	**9.50±0.40^{a}**	0.60±0.21	0.74±0.34	0.39±0.19	3.41±0.67	3.32±1.12
		TRI2024	3.10±0.10	9.22±0.20^{ab}	0.62±0.17	0.64±0.16	0.37±0.13	7.56±4.61	5.49±2.27
		AT53	3.75±0.10	9.02±0.20^{ab}	0.33±0.03	0.49±0.11	0.21±0.07	3.64±0.68	7.68±3.86
		TV9	3.45±0.10	7.49±0.60^{b}	1.01±0.46	0.38±0.06	0.49±0.28	4.16±0.85	9.00±3.61
		Mean	3.50±0.10	8.81±0.30	0.64±0.14	0.56±0.09	0.36±0.08	4.72±1.14	6.37±1.41
		p-value	0.294	**0.031***	0.412	0.594	0.762	0.586	0.564
Highland	Young leaf	BC1248	4.46±0.10	8.35±0.80	11.1 ± 1.00	2.20±0.10	1.28±0.09	0.057±0.00	0.38±0.08
		TRI2024	4.47±0.30	7.98±0.20	12.2 ± 0.50	2.42±0.10	1.59±0.09	0.057±0.01	0.30±0.05
		AT53	5.18±0.10	6.74±1.00	10.3 ± 1.40	2.63±0.20	1.74±0.16	0.057±0.01	0.36±0.07
		TV9	4.31±0.60	5.78±0.50	10.8 ± 0.50	2.52±0.30	1.46±0.02	0.050±0.00	0.44±0.07
		Mean	4.60±0.20	7.22±0.40	11.08±0.50	2.44±0.10	1.51±0.07	0.055±0.00	0.37±0.03
		p-value	0.309	0.090	0.592	0.366	0.069	0.802	0.574
	Mature leaf	BC1248	3.81±0.10	5.65±0.20	6.66±0.50^{b}	7.21±0.70	1.91±0.26	0.073±0.01	2.04±0.27
		TRI2024	3.69±0.10	5.88±0.50	7.51±0.20^{ab}	6.63±0.30	2.22±0.24	0.080±0.00	1.59±0.08
		AT53	4.08±0.10	6.70±1.00	**8.75±0.20^{a}**	7.82±0.80	2.57±0.28	0.107±0.01	2.32±0.05
		TV9	4.06±0.10	4.44±0.30	7.79±0.20^{ab}	8.40±0.80	2.25±0.06	0.093±0.01	2.20±0.43
		Mean	3.91±0.10	5.67±0.40	7.68±0.30	7.52±0.40	2.24±0.10	0.088±0.01	2.04±0.14
		p-value	0.148	0.138	**0.009****	0.354	0.312	0.101	0.272

Mean ± SE with different superscript letter is significantly different at $p < 0.05$ using Tukey, significance of the values: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 3
Antioxidant activity (AOA) of young and mature tea leaves from lowland and highland plantations of Malaysia.

Plantation	Clone	TPC (mg GAE/g)		DPPH IC50 (μg/mL)		FRAP (mM Fe (II)/g)	
		Young	Mature	Young	Mature	Young	Mature
Lowland	BC1248	19.60±0.15	18.80±0.14	**50.70±1.86[b]**	**72.30±0.25[c]**	2.10±0.14	2.20±0.15
	TRI2024	19.30±0.30	18.10±0.26	74.30±2.79[a]	118.00±3.01[b]	1.99±0.06	2.09±0.06
	AT53	19.30±0.26	15.90±1.85	74.40±4.99[a]	221.00±17.10[a]	1.55±0.16	1.63±0.17
	TV9	19.30±0.04	18.20±0.47	73.0 ± 4.31[a]	110.00±4.08[bc]	1.86±0.18	1.95±0.19
	Mean	19.38±0.10	17.74±0.53	68.29±3.45	130.29±16.99	1.88±0.09	1.97±0.09
	p-value	0.607	0.243	**0.004****	**0.000*****	0.114	0.115
Highland	BC1248	18.10±1.20	17.00±0.54	140.00±1.15	133.00±1.80[a]	**1.49±0.08[a]**	1.29±0.12
	TRI2024	16.20±0.37	16.80±0.55	141.00±2.38	**127.00±1.16[b]**	1.09±0.09[ab]	1.36±0.18
	AT53	18.10±0.63	17.70±0.22	133.00±3.53	135.00±0.60[a]	0.87±0.10[b]	0.97±0.13
	TV9	17.50±0.72	16.10±0.17	132.00±2.34	134.00±0.82[a]	1.12±0.20[ab]	1.46±0.08
	Mean	17.47±0.41	16.91±0.25	136.39±1.64	132.52±1.03	1.14±0.09	1.27±0.08
	p-value	0.359	0.116	0.064	**0.007****	**0.048***	0.115

Mean ± SE with different superscript letter is significantly different at $p < 0.05$ using Tukey, significance of the values: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 4
Two-way ANOVA test F-value of tea foliar nutrient concentration affected by leaf age, clone and altitude in Malaysia.

Leaf age	Factors	Foliar nutrient concentration						
		N	P	K	Ca	Mg	Fe	Al
Young leaves	Clone	0.32	1.86	0.55	2.12	11.92***	29.11***	153.80***
	Altitude	11.01**	73.28***	464.53***	388.59***	296.42***	1210.55***	1201.90***
	Clone x Altitude	2.2	5.78	2.11	1.07	2.95	29.10***	157.90***
Mature leaves	Clone	2.59	5.67**	3.96*	0.80	0.53	0.68	0.82
	Altitude	9.23**	75.84***	1191.04***	384.89***	161.01***	14.96**	8.70**
	Clone x Altitude	0.72	0.80	5.92	1.62	1.47	0.69	0.64

Significance of the values is indicated as follows: ns = not significant, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 5
Two-way ANOVA test F-value of tea AOA affected by leaf age, clone and altitude.

Leaf age	Factors	Antioxidant activity (AOA)		
		TPC	DPPH	FRAP
Young leaves	Clone	1.41	4.97*	6.27**
	Altitude	21.90***	931.92***	58.22***
	Clone x Altitude	0.98	11.19***	0.41
Mature leaves	Clone	0.79	51.66***	4.67*
	Altitude	2.55	0.25	48.84***
	Clone x Altitude	2.93	48.42***	0.76

Significance of the values is indicated as follows: ns = not significant, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

The PCA of young (Fig. 4a) and mature leaves (Fig. 4b) depicted that tea clones were clustered into two groups. Clones 1 – 4 were from the lowland plantation and were significantly associated with the AOA, foliar P, Fe and Al. Another cluster consists of clones with numbers 5 – 8 originating from the highland plantation and found to be highly associated with foliar K, Ca and Mg.

4. Discussion

4.1. Foliar nutrient concentration

4.1.1. The effects of altitudes and clone types

The foliar nutrient concentration of tea was affected by various factors, including altitude, clone type and leaf age. Altitude was the most significant factor affecting foliar nutrient concentrations across all clone types and leaf ages. Compared to the lower elevation, clones grown in highland plantations have higher foliar nutrient content. Our results demonstrated that tea clones grown in highland plantations exhibited higher foliar nutrient concentrations compared to those cultivated at lower altitudes. However, this increase was not consistent across all nutrients, as not all elements responded proportionally to altitude. Specifically, we observed that tea leaves' concentrations of N, K, Ca and Mg tend to increase with altitude. This finding was consistent with previous studies. For instance, Xiang et al. (2021) reported that foliar N and P concentration, along with leaf C:N ratio, increased significantly with altitude. Several researchers have suggested that this trend may result from declining biomass production at higher altitudes. This was probably attributed to cold-induced growth limitations in plants (Jeyakumar et al., 2020).

Plants growing at high altitudes generally experience slower growth rates than those at lower altitudes. This is influenced by environmental factors such as lower temperatures, reduced nutrient availability, and decreased photosynthetic efficiency (Jeyakumar et al., 2020). It is known that for every 1 km increase in elevation, temperature drops by approximately 6.5 °C. These colder conditions reduce soil microbial and enzymatic activity, limiting nutrient mineralization and availability (Xu et al., 2015). Moreover, decreased air density and atmospheric pressure at high altitudes result in lower CO_2 concentrations and slower transpiration rates. This will further reduce photosynthetic rates (Wang et al., 2017). As a result, it was hypothesized that the accumulation of nutrients in tea leaves at higher altitudes may reflect the plants' limited ability to utilize the absorbed nutrients for growth rather than increased uptake alone.

Nutrient concentrations were varied among young and mature leaves of lowland tea clones. On the other hand, nutrient concentrations were only varied among mature leaves of highland tea clones. Clone AT53 was considered the best among the four clones evaluated since it consistently showed higher foliar nutrient concentrations for young and mature leaves across both plantations. This result was similar to Amirah et al. (2023b), in which AT53 outperformed six others in terms of foliar nutrient concentrations. Clone AT53 had the highest levels of K, Mg, Fe and Al, while clones 2026 and 663 displayed the highest N and P, respectively. In addition, we also discovered that AT53 demonstrated higher foliar nutrient concentration in highland plantations.

The order of nutrient elements availability of young leaves' lowland plantation was as follows: $N > Al > P > Fe > K > Ca > Mg$. Meanwhile, mature leaves in lowland plantations also follow a less similar pattern,

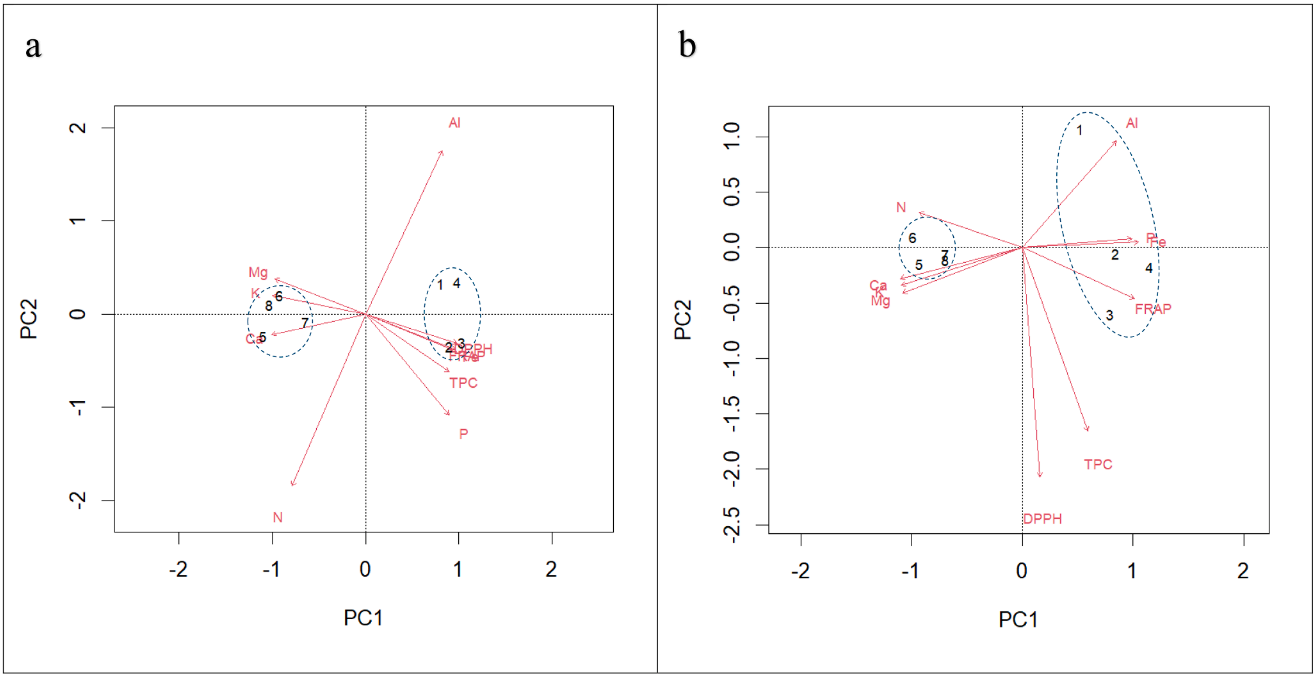

Fig. 4. PCA biplot plant model foliar nutrient concentration and antioxidant activity in a) young and b) mature tea of BC1248, TRI2024, AT53 and TV9 (Note: 1–4 = lowland clones, 5–8 = highland clones).

Table 6
Statistic of PCA axis related to leaf ages from lowland and highland plantations in Malaysia.

	Leaf Ages					
	Young Leaves			Mature Leaves		
	PC1	PC2	PC3	PC1	PC2	PC3
Variance (%)	0.84	0.07	0.04	0.69	0.19	0.05
Cumulative (%)	0.84	0.91	0.95	0.69	0.88	0.92
N	0.71	0.46	0.29	-0.75	0.13	0.45
P	-0.81	0.27	-0.26	0.81	0.04	0.31
K	0.90	-0.05	-0.09	-0.89	-0.15	-0.09
Ca	0.91	0.05	0.008	-0.90	-0.12	-0.10
Mg	0.88	-0.10	0.06	-0.88	-0.17	-0.09
Fe	-0.87	0.10	-0.06	0.86	0.02	-0.19
Al	-0.74	-0.44	0.24	0.70	0.41	-0.03
TPC	-0.81	0.16	0.31	0.48	-0.71	0.16
DPPH	-0.89	0.08	0.10	0.13	-0.89	-0.01
FRAP	-0.84	0.09	-0.086	0.83	-0.20	-0.05

Note: PC1 = Principal Component 1, PC2 = Principal Component 2, PC3 = Principal Component 3.

with P and Al being the second and third highest nutrient elements, respectively. This was less similar to the work of Amirah et al. (2023b), which involved a more significant number of clones from lowland plantations. In addition, the sequence of nutrient elements in young leaves from highland plantations was observed as follows: $N > K > P >$ Ca > Mg > Fe > Al. In contrast, mature leaves from the same plantations exhibited a slightly different pattern, with Ca and P occupying the third and fourth positions and Al and Fe shifting to the sixth and seventh positions, respectively. Based on the nutrient element availability order across both plantations, we found that N was the highest nutrient element available in tea leaves. This suggests that N is essential for tea growth and development (Tseng & Lai, 2022). Nitrogen significantly influences the growth of both roots and shoots. It also affects the levels of various endogenous hormones, including indoleacetic acid, gibberellin and zeatin, which are crucial for plant growth (Xiao et al., 2018). It also enhances the content of amino acids, particularly L-theanine, which improves tea quality (Chen et al., 2021). Nitrogen input is directly proportional to tea yield (Sitienei et al., 2013), with approximately 3.88 g/kg/pot or 225 kg/ha/year being effective for achieving high yields (Xiao et al., 2018). Combining N with other nutrients such as P and K can enhance tea growth and yield (Debere et al., 2014).

We found that lowland-grown clones possessed higher foliar P. We hypothesized that it was probably due to higher application of P fertilizer rates in the lowland (200 kg ha^{-1} year^{-1}) (Amirah et al., 2023b) over the highland plantation (100 kg ha^{-1} year^{-1}). In contrast, the highland tea clones had higher foliar K concentration. This was similar to the study conducted by Tseng and Lai (2022). Potassium is the second macronutrient needed by tea; however, with the sufficiency levels of 15 – 20 mg g^{-1}, there were no clones in both plantations considered sufficient for foliar K concentration. Sufficient K will improve tea metabolism and enhance biotic and abiotic resilience by stimulating and modulating various enzymes, thus substantially boosting the growth and development of tea (Huang et al., 2022; Ruan et al., 2013). Plants generally absorb more potassium compared to other elements, except for N and, in particular circumstances, Ca (Hawkesford et al., 2011). This is similar to our finding, especially in lowland plantations, where the K was ranked as the fourth macronutrient after Ca.

Our results demonstrated that highland-grown tea possessed higher Ca concentration compared to the lowland. This is likely attributed to several factors, such as soil composition, environmental stress, plant physiology and adaptation. Highland areas often have soils with higher Ca content. This is due to calcic horizons or specific geological formations like limestone or dolomite, rich in Ca. This geological background contributes to the higher availability of Ca in the soil, which the plants absorb (Li et al., 2020). In addition, plants in highland areas may experience more environmental stress, such as lower temperatures and higher UV radiation. This can lead to an increase in Ca uptake as a protective mechanism. It plays a crucial role in stabilizing cell walls and membranes, which helps plants cope with stress (El Habbasha & Ibrahim, 2015). Formation of Ca-pectate in the middle lamella of cells to maintain cell structure and functions is a part of plant physiology and adaptation of plants (Proseus & Boyer, 2012).

Other macronutrients, such as Mg, have been established to have significant roles in plants since Mg promotes chlorophyll synthesis and acts as a catalyst for many enzymatic reactions (de Bang et al., 2021;

Zhang et al., 2021). In contrast with Ca and Mg, we found that more foliar Fe and Al were available in lowland clones. This is probably due to the higher foliar P in clones grown in lower elevations over highland plantations, supported by the similar arrow direction in the PCA result. Iron is an important micronutrient since it is involved in metabolic activities such as DNA synthesis, respiration, and photosynthesis. Meanwhile, Al is toxic to most plants, even in micromolar doses. Nevertheless, tea is an acidophilic plant that grows well in acidic soils and takes up vast amounts of Al in their shoots (Amirah et al., 2023b; Carr et al., 2003), similar to *Melastoma malabathricum* (Khairil & Burslem, 2018; Mahmud et al., 2024).

In addition to altitudes, nutrient concentrations in tea are significantly influenced by the type of clones used. Our study identified clone AT53 as a superior genotype with the highest foliar K concentration in lowland and highland plantations. In lowland plantations, this clone also recorded the highest foliar Mg, Fe, and Al. These results aligned with the findings of Amirah et al. (2023b), who conducted a similar study focused on tea in the lowland regions. This was attributed to the similar genetic diversity among the Malaysian tea clones evaluated. A recent study by Wang et al. (2024) reported variation in nitrogen use efficiency (NUE) among six clones evaluated. Clone TC12 exhibited the highest NUE, while LJCY had the lowest. This variation is linked to the differential expression of genes involved in nitrogen uptake metabolism, such as AMT1.2, NRT2.4, NRT3.2 in roots and AAP6 and AAP7 in stems and shoots. Ruan et al. (2019) investigated the responses of two genotypes (Longjing43/LJ43 and Liyou002/LY002) to nitrogen spatial heterogeneity. The result showed that LJ43 exhibit greater adaptability to uneven N distribution than LY002. Gene expression analysis revealed that LJ43 upregulated key genes involved in amino acid transformation and N assimilation, including CsGADs, CsNRT2.5 and CsGS2.

4.1.2. The effects of leaf ages

Leaf ages also influenced tea foliar nutrient concentrations. Our study demonstrated that N concentration tends to be higher in young leaves than in mature ones. This was because young leaves are actively growing and require more nitrogen to synthesise proteins and other essential compounds. On the other hand, mature leaves generally have lower concentrations as N was remobilized to support the growth of new shoots (Ruan & Gerendás, 2015; Zaman et al., 2022; Zou et al., 2024). Like N, foliar P and K are more concentrated in young leaves. Phosphorus is critical in energy transfer and nucleic acid synthesis, which are vital for young leaves' rapid growth and development (Yan et al., 2024; Zou et al., 2024). Meanwhile, potassium was essential for various physiological processes, including enzyme activation and osmoregulation, which are crucial during the early stages of leaf development (Ruan & Gerendás, 2015; Zaman et al., 2022)

Higher foliar Ca and Mg concentration in mature leaves over younger tea leaves in our study was similar to the result demonstrated by Carr et al. (2003) and the most recent result by Pongrac et al. (2020). Results from Carr et al. (2003) revealed that, on average, young tea leaves contained 1.72 and 3.56 mg g^{-1} Mg and Ca, while mature leaves may contain higher Mg and Ca at 2.6 and 18.7 mg g^{-1}, respectively. In addition, Pongrac et al. (2020) investigated the relative distribution of Mg, Ca and Mn in tea leaves using micro-PIXE. They found that the concentrations of Mg, Ca and Mn increased from young to mature leaves by 1.7, 9.4 and 8.1-fold, respectively.

In contrast to other nutrients, the Fe concentrations have fluctuated across both plantations. Young tea leaves of lowland tea clones contained higher Fe than mature leaves. However, in highland plantations, Fe were higher in mature tea leaves. The highland result was similar to previous studies of Zaman et al. (2022), which indicated that Fe concentration was higher in mature than young tea leaves. According to Zaman et al. (2022), the average Fe concentration in mature leaves ranged from 0.33 – 0.57 mg/g, whereas in young leaves, it ranged from 0.04 – 0.2 mg/g. Our lowland result of Fe concentration (young > mature leaves) was contrary to Zaman et al. (2022). We hypothesized that it was due to a combination of more active root systems and a biochemical environment that supports efficient Fe absorption. Roots of tea plants are efficient in forming Fe / iron plaques, which facilitate Fe uptake, especially under acidic conditions (Zhang et al., 2019). This was supported by the soil nutrient result of lowland plantation, with an average pH of around 4.2 and Fe concentration in soil ranging between 6.12 – 10.22 mg/g (Amirah et al., 2023b). Young tea leaves have a different biochemical composition from mature ones. They contain higher levels of certain compounds like catechins and amino acids, associated with higher metabolic rates and nutrient uptake. This biochemical environment supports more efficient Fe uptake (Samanta et al., 2016).

The Al concentrations have fluctuated across both plantations, similar to Fe. Young tea leaves of highland tea clones contained lower Al than mature leaves. However, in lowland plantations, Al was higher in young tea leaves. We found that most clones grown in lowland plantations had up to 2.95-fold higher foliar Al content in young leaves than in mature leaves, except for TV9. This result differs from the findings of previous studies by Carr et al. (2003); Ruan et al. (2006), and Zhang et al. (2018b), in which old tea leaves accumulated a higher amount of Al than younger tea leaves. The most recent study by Yang et al. (2022) showed that the Al concentration tended to increase with the position of the lower (older) leaves. The average Al concentrations for mature and young leaves rose by 2.7-fold and 1.9-fold among all varieties during the winter and summer months, respectively. Moreover, Al concentrations in the remaining leaves (4th to 11th leaves) rose by 1.5–fold in a subsequent manner.

The Al deposit mechanism in tea is fairly complicated and is related to several factors, such as altitude, agronomic practices and physicochemical properties of the soil (Zhang et al., 2018b). Altitude affects Al accumulation through humidity, which influences transpiration and mineral distribution in plants. Reduced transpiration in highland tea clones leads to lower accumulation compared to lowland clones (de Silva et al., 2016; Shen & Ma, 2001). Agronomic practices, particularly the overuse of N fertilizer can significantly impact Al levels. The N fertilizers contribute to soil acidification, which increases Al bioavailability and uptake by tea plants (Fang et al., 2014; Qiao et al., 2018). Soil physicochemical properties are also one of the factors of higher Al uptake in young tea leaves of lowland tea clones. We hypothesized that soil pH and texture were among the factors influencing high Al uptake in young tea leaves at lowland plantations. The acidic pH (4.2) of the lowland tea plantation soil influences the increase of Al bioavailability in soil (Amirah et al., 2023b). The clay soil texture retains nutrients due to its high CEC; it also binds P and other essential nutrients, making them less available to plants (Qu et al., 2021), which may inadvertently lead to an increase in Al uptake as an alternative. Therefore, reducing application of fertilizers (N) and applying biostimulants during cultivation is one of the important agronomic practices to alleviate the accumulation of Al in tea plantation soil (Abbott et al., 2018; Wang, et al., 2020; Wong et al., 2020).

4.2. Antioxidant activity

4.2.1. The effects of altitudes and clone types

Tea antioxidant activity assay consisted of TPC, DPPH and FRAP. DPPH and FRAP assay; measuring the tea samples' free radical scavenging activity (antioxidant activity) and electron-donating potential, respectively. Meanwhile, the TPC measures the overall amounts of phenolic compounds in the tea samples evaluated. Our TPC content ranged from 16.91 – 19.38 mg GAE/g, which approximated to about 1.6 – 1.9 %. This result was lower than that of Prawira-Atmaja et al. (2018) and Yadav et al. (2020), which ranged between 12.12 – 14.59 % and 59 – 57 %, respectively. We hypothesized that the primary factor contributing to lower polyphenol content in our extraction method was the sample preparation approach. Our method involved oven-drying fresh tea leaves at 60 °C for four days before extraction (Amirah et al., 2023b).

Conversely, both Prawira-Atmaja et al. (2018) and Yadav et al. (2020) utilized fresh leaves directly. Thus any prolonged thermal treatments might cause some degradation of the heat–sensitive polyphenols through oxidation, polymerization, and thermal decomposition processes (Geng et al., 2023; Jiang et al., 2023; Unnadkat & Elias, 2012).

The extraction methodologies differ substantially in complexity and efficiency. Our approach was using a single-step ultrasonic-assisted extraction (UAE) at 40 °C for 30 min. In contrast, Yadav et al. (2020) employed a multi-step process involving hot steeping at 65 °C, mechanical homogenization, and sequential re-extraction. Meanwhile, Prawira-Atmaja et al. (2018) utilized a comprehensive approach combining manual grinding, hot methanol extraction, thermal maceration at 60 °C for 2 h, and ultrasonic treatment. These comprehensive methods provide superior extraction efficiency through extended extraction times (Casazza et al., 2012; Khamtache-Abderrahim et al., 2021) and multiple extraction mechanisms. The multiple extraction method leverages the strengths of various techniques and solvents to maximize polyphenol extraction (Linhares Sabino et al., 2025). The cumulative impact of these methodological differences explains the order-of-magnitude difference in polyphenol content observed in our study. The combination of thermal sample degradation during drying, lower extraction temperature, shorter extraction time, lack of mechanical cell disruption, and single-step versus sequential extraction creates a multiplicative effect on polyphenolic recovery. These factors collectively could reduce polyphenol extraction efficiency by up to 90 %.

In this study, the AOA of tea was also affected by various factors, including altitude, clone type and leaf age. Altitude was the most significant factor affecting AOA across all clone types and leaf ages. We found that clones grown at a lower elevation tend to have a higher antioxidant activity (AOA). This was similar to earlier studies in Taiwan (Chen et al., 2014) and Indonesia (Martono et al., 2016). Both authors reported that cultivation altitude was inversely correlated with total phenolic. In Malaysia, previous studies concerning the AOA of tea have been conducted (Amirah et al., 2023b; Chan et al., 2007). Interestingly, the results from this study contrasted against the earlier lowland and highland results; demonstrating that tea grown in lower elevations tends to have higher AOA. This was supported by higher FRAP content and lower DPPH IC_{50} values.

Higher AOA in the lowland tea plantations is associated with the plant's elevated stress response to multiple stressors characteristic of lowland conditions, including light intensity. At higher altitudes, although UV radiation is more intense due to a thinner atmosphere. However, overall light intensity is more diffuse because of frequent cloud cover, mist and lower air temperatures (Kómar & Nečas, 2023; Tomanová & Pokorná, 2021). In contrast, although the atmosphere is denser at lower altitudes, the direct solar irradiance can be more intense, particularly under clear skies and longer sun exposure. This leads to potential photooxidative stress (Koc & Cam, 2020; Marion, 2020). When exposed to high light intensity (particularly >1000 μmol m^{-2} s^{-1}), tea plants may increase polyphenols such as catechins and epicatechins, as protective compounds. Additionally, flavonoid biosynthesis, including flavonols and anthocyanins, is rapidly activated under high light, contributing to photoprotection and antioxidative defense (Zagoskina et al., 2023; Zhang et al., 2022).

In lowland environments, high light intensity typically occurs alongside elevated temperature, with the critical threshold triggering heat damage to tea plants was 30.1 °C (Wang et al., 2023b). This heat stress can impair physiological growth patterns, biochemical composition, and sensory attributes (bitterness, astringency and aroma) of tea (Xie, 2024). When combined, high light intensity and temperature accelerate evapotranspiration, causing greater water loss from both soil and plant surfaces, potentially intensifying water scarcity. As a result, tea plants in these regions often face drought stress, one of the major abiotic factors that disrupts physiological processes (Costa et al., 2007; Grunwald et al., 2024). This stress can reduce the production of protective compounds like pathogenesis-related proteins, making plants more susceptible to biotic attacks. Meanwhile, warmer and more humid microclimates in lowlands tend to favor the proliferation of insect pests and pathogenic fungi or bacteria (Majeed et al., 2025).

Collectively, the combination of all the stressors in the lowland environment imposes significant physiological stress on tea plants. These combined stresses trigger oxidative damage through the excessive production of reactive oxygen species (ROS) (Dumanović et al., 2020; You & Chan, 2015; Wan et al., 2024). In response, the plants activate various defense mechanisms, particularly the upregulation of antioxidant systems. One of the primary strategies involves enhancing polyphenol synthesis, which functions as a potent antioxidant to neutralise ROS. Polyphenols also help reinforce cell walls and protect against pathogen attacks (Dumanović et al., 2020; Tariq & Ahmed, 2024). As a result, lowland-grown tea typically exhibits higher AOA than tea grown in highland regions. Meanwhile, high-altitude grown tea has a more complex and pleasant aroma profile due to the presence of volatiles such as linalool and geraniol (Wang et al., 2023b). Moving forward, combining teas from both altitudes can yield a synergistic blend that merges high antioxidant content with complex aroma profiles.

Despite the altitude, distinct clone types also influenced the AOA of tea. We found that clone BC1248 is a potential clone with the highest AOA across both plantations. This finding was similar to the result of Amirah et al. (2023b) whereas the AOA of clone BC1248 outperformed six other lowland tea clones. Other tea researchers working in other geographical regions also had similar reports. For instance, Benti et al. (2022) revealed that clone BB-35 had the highest caffeine content, while clone 6/8 had the highest polyphenol content and AOA among the five Ethiopian clones evaluated. Clone TS520 exhibited abundant antioxidant compounds and had exceptional AOA performance (DPPH, ABTS and total antioxidant activity assay) among four Indian clones tested (Das et al., 2024). Similarly, clone Iran 100 showed the highest total phenolic and flavonoid content among the 12 high-yielding clones in Iran (Gonbad et al., 2015).

Genetic diversity among tea clones significantly influences the variation in tea AOA (Amirah et al., 2023b; Benti et al., 2022; Das et al., 2024; Gonbad et al., 2015). Genetic diversity was observed in tea clones from Northeast and Southern India, with genetic variance within populations (Negi et al., 2019). Similarly, a study in tea germplasm in Uganda revealed high within-population variance, indicating significant diversity (Tadeo et al., 2024). Different tea clones possess distinct genetic compositions. This leads to variations in the levels of key antioxidant compounds, such as catechins, theaflavins and polyphenols. Earlier studies have shown that different tea clones possess unique genetic markers that correlate with catechin levels and other antioxidants. For instance, specific gene regions called QTLs (Quantitative Trait Loci) have been linked to tea plants' catechin content and antioxidant activity (Hazra et al., 2020). The QTL can be used as a marker to identify and select tea clones with high catechin and antioxidant levels. Additionally, variations in gene expression related to phenolic metabolism, enzyme activity, and stress responses contribute to differences in antioxidant potential.

Environmental factors, such as different altitudes, along with distinct light intensities and temperature, interact with genetic traits, further influencing AOA. We found that the interaction between altitude and clone type significantly affected tea AOA ($p < 0.05$). This suggests that genetic and environmental factors play crucial roles in determining the AOA of tea. For instance, tea research across Bangladesh reveals significant variations in antioxidant properties among Bangladesh Tea (BT) and Tocklai Vegetative (TV) clones, with certain clones demonstrating strong therapeutic potential. Similarly, green teas from various tea gardens and clones depicted variations in physicochemical traits and bioactivity, influenced by genetic and environmental factors (Sarkar et al., 2022, 2023). Additionally, inoculation with arbuscular mycorrhizal fungi significantly enhanced tea plant growth, nutrient uptake, and the accumulation of beneficial secondary metabolites (Sarkar et al., 2020). These studies collectively emphasize how cultivation conditions, genetic factors and biological associations significantly influence the beneficial properties of tea. Regional variations in growing conditions contribute to unique biochemical profiles in otherwise genetically similar tea clones.

Similarly, lowland-grown clones have advantages over highland clones with suitable light intensities and warmer temperatures for improving catechin synthesis. However, tea clones may thrive at higher altitudes through a distinct mechanism from lowland tea clones. Light exposure significantly influences flavonoid metabolism in tea plants. Genes encoding flavonoid synthase are highly associated with the light-induced accumulation of flavonoids, essential in protecting against photooxidative stress (Zhang et al., 2022). Additionally, genes involved in the flavonoid biosynthesis pathway, such as PAL, C4H and 4CL are upregulated under stress conditions, enhancing the antioxidant potential of tea (Ren et al., 2021). Key antioxidant enzymes like CAT, SOD and peroxidase help manage oxidative stress by increasing their activity under various stress conditions (light, heat or salt stress), boosting the AOA of tea plants (Zhang et al., 2022; Zhang et al., 2024).

4.2.2. The effects of leaf ages

Leaf ages also affected tea AOA from both plantations. We found that young tea leaves have better AOA performance than mature leaves. This was supported by the higher TPC and lower DPPH IC_{50} value. This result was similar to a previous study conducted by (Dorkbuakaew et al. (2016); Liu et al., 2020b). In Malaysia, Chan et al. (2007) and Izzreen et al. (2013) have studied the leaf age effect on tea AOA and ranked the levels of AOA as follows: shoot > young leaves > mature leaves. Young tea leaves contain higher levels of total phenolic and total flavonoid content, which are major contributors to the AOA of tea. Earlier studies have shown that the AOA, as measured by various assays such as DPPH and FRAP, was significantly higher in young leaves due to their elevated TPC and TFC levels (Chan et al., 2007; Dorkbuakaew et al., 2016; Fatanah et al., 2016; Izzreen et al., 2013).

Some physiological changes occurred during leaf maturation. Upon maturation, the leaf undergoes physiological changes, such as reduced moisture and TPC. Tea leaves lost a substantial volume of water throughout maturation, ranging from 72.53 % to 58.23 %. Along with water loss, phenolic content has been reported to decline after maturation for both young and old tea leaves, dropping from 18.82 % and 9.52 %, respectively (Liu et al., 2020b). Tea phenolic profiles were also dramatically altered upon leaf aging. During leaf maturation, there is a process of degalloylation, where the galloylated catechins (flavonols), such as ECG and EGCG, decrease. In contrast, other phenolic compounds, such as flavonols and cell wall-bound phenolics, increased (Liu et al., 2020b; Samanta et al., 2016). Although delivering less potency in some antioxidant assays, flavonols can still contribute significantly to FRAP activity due to their ability to reduce ferric ions (Dorkbuakaew et al., 2016). In addition, mineral content has a potential relationship with tea's antioxidant activity. Certain minerals, such as iron (Fe) and copper (Cu) are known to play a role in redox reactions and can enhance the overall reducing power measured by FRAP assay. Iron is crucial in cell redox systems and various enzymes (Cakmak et al., 2023). The FRAP assay directly measures Iron's ability to alternate between Fe^{2+} and Fe^{3+} states, reflecting its reducing power. Like iron, copper's redox activity can be quantified using the FRAP assay, contributing to the overall antioxidant capacity (Berker et al., 2007). Therefore, these explain why mature leaves exhibited higher FRAP values than young leaves in our study despite the contrasting TPC result.

5. Conclusion

The nutrient elements and AOA of young and mature tea leaves were associated with altitude and clone type under tropical conditions. This study demonstrated that altitude and tea clone types significantly influence foliar nutrient elements and AOA in young and mature leaves. Young leaves consistently exhibited higher nutrient concentration and AOA than mature leaves across AT53, BC1248, TRI2024 and TV9 clones. The AT53 clones showed higher nutrient availability in highland plantations, while the highest AOA was observed in young leaves of BC1248 clones at lowland plantations. The principal component analysis identified lowland regions in tropical Malaysia as the more suitable area for cultivating tea with higher AOA across all clones. These findings offer valuable insight for tea growers in selecting the appropriate leaf age, altitude and clone type to enhance sustainable cultivation practices and improve tea quality with high antioxidant activity.

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CRediT authorship contribution statement

Wisnu Eko Murdiono: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Nur Amirah Syafiqah Salman:** Investigation, Formal analysis, Data curation. **Nor Asma Ab Razak:** Writing – review & editing, Supervision, Resources, Methodology. **Mohd. Izuan Effendy Halmi:** Resources, Methodology, Conceptualization. **Jean Wan Hong Yong:** Writing – review & editing, Methodology, Funding acquisition. **Abbe Maleyki Mhd. Jalil:** Resources, Conceptualization. **David F.R.P. Burslem:** Writing – review & editing, Visualization, Conceptualization. **Khairil Mahmud:** Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization, Writing – review & editing, Writing – original draft.

Declaration of competing interest

The authors declared that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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