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SCREENING OF INDONESIAN SINENSIS TEA CLONES (*Camellia sinensis* var. sinensis) FOR HIGH-QUALITY GREEN TEA: EMPHASIS ON INDONESIAN NATIONAL STANDARDS COMPLIANCE, HIGH PROTEIN, AND LOW CAFFEINE

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ABSTRACT

Tea is one of the oldest beverages known to humanity, with green tea being among the most popular types due to its distinctive flavor and health benefits. The development of green tea is essential, both in terms of sensory quality and functional value. This study aimed to screen 35 Indonesian Sinensis tea clones to identify those with superior flavor, compliance with Indonesian National Standards (SNI), high protein content, and low caffeine levels. The research was conducted by processing fresh tea shoots into green tea, followed by quality evaluation based on SNI parameters, including moisture content, total ash content, crude fiber content, and polyphenol content, as well as analysis of protein and caffeine levels. The experiment employed a Randomized Complete Block Design (RCBD) with two replications and 35 clones as treatments. The results revealed significant variation in quality among the tested clones. Clone I.2.85 and II.2.108 was identified as the best-performing clone, meeting SNI quality standards (moisture content, total ash, crude fiber, and polyphenols), exhibiting high protein levels, low caffeine levels, and a premium flavor profile.

Key words: Green tea quality, Screening, Sinensis tea clones

INTRODUCTION

Tea (*Camellia sinensis* L.) is globally recognized as one of the most prominent functional beverages, with green tea being particularly valued for its distinct sensory profile and profound health benefits. The biochemical composition of fresh tea leaves, primarily determined by the genetic traits of the specific cultivar, fundamentally dictates the quality of the final green tea product. Recently, global tea breeding programs have increasingly focused on developing clones with high concentrations of proteins and free amino acids, which contribute to the desirable "umami" flavor and mitigate astringency (Zhu *et al.*, 2023). Simultaneously, there is a rising consumer demand for low-caffeine functional beverages to prevent the adverse physiological effects associated with high caffeine intake, such as insomnia and cardiovascular palpitations (Jin *et al*., 2022). Consequently, cultivating *sinensis* varieties (*Camellia sinensis* var. *sinensis*) that naturally express both high protein and low caffeine levels has become a paramount global effort in the premium green tea industry.

Despite the ideal agro-climatic conditions for tea cultivation in Indonesia, the national tea industry faces a significant challenge: plantations are historically dominated by the *assamica* variety (*Camellia sinensis* var. *assamica*). While *assamica* is highly productive and optimal for robust black tea, it is generally unsuitable for producing premium, delicate green tea (Widiastuti & Nafi'ah, 2021). To revitalize the green tea sector, the introduction and cultivation of local *sinensis* clones are urgently required. However, the commercialization of any new clone in Indonesia must strictly adhere to the quality and safety parameters mandated by the Indonesian National Standards (Standar Nasional Indonesia/SNI) for green tea, which serves as the fundamental baseline for market acceptance (Rahmawati *et al.*, 2024).

The current state of the art in Indonesian tea research has predominantly focused on morphological characterization, yield optimization, and general adaptability of existing commercial clones. Previous studies evaluating green tea quality have largely been restricted to assessing basic polyphenol content and total antioxidant capacity (Zhao *et al.*, 2022). A critical gap remains in the literature, as there is a profound lack of comprehensive screening programs targeting the specific biochemical traits required for modern premium green tea. Previous findings have rarely integrated rigorous SNI compliance with advanced biochemical profiling specifically aimed at identifying the dual traits of high protein and low caffeine within a large population of local *sinensis* clones. This weakness hinders the strategic selection of superior local genotypes necessary to compete in the high-value global market.

To address this problem, this research employs a systematic screening approach evaluating the biochemical and standard compliance profiles of a vast collection of local genetic resources. The novelty of this research lies in its comprehensive, multi-parameter evaluation that bridges national regulatory compliance (SNI) with advanced consumer-driven biochemical targets (high protein and low caffeine) within native Indonesian *sinensis* populations—an integration that has not been previously established. The contribution of this study will provide crucial genetic baselines for future clonal propagation and commercial release. Therefore, this study explicitly aimed to screen 35 Indonesian *sinensis* tea clones to identify superior genotypes that strictly comply with the Indonesian National Standards (SNI) while exhibiting the exceptional traits of high protein content and low caffeine levels for high-quality green tea production.

MATERIALS AND METHODS

This study utilized 35 *sinensis*-type tea clones obtained from the collection of the Research Institute for Tea and Cinchona (Table 1). The experimental stages included green tea processing, quality evaluation based on Indonesian National Standard (SNI) parameters (comprising moisture content, total ash content, crude fiber content, and polyphenol content), as well as the analysis of protein and caffeine levels.

Table 1. Tea Clones Used in the Study

No.	Clones	No.	Clones	No.	Clones	No.	Clones
1.	I.1.70	10.	II.1.3	19.	II.3.16	28.	S3
2.	I.1.93	11.	II.1.32	20.	II.3.38	29.	SGMBA
3.	I.1.100	12.	II.1.38	21.	II.4.149	30.	YABUKITA

4.	I.1.101	13.	II.1.60	22.	II.4.32	31.	GMBS 1
5.	I.2.34	14.	II.1.76	23.	II.4.178	32.	GMBS 2
6..	I.2.45	15.	II.1.98	24.	R1	33.	GMBS 3
7.	I.2.85	16.	II.2.43	25.	R3	34.	GMBS 4
8.	I.2.188	17.	II.2.108	26.	S1	35.	GMBS 5
9.	I.4.113	18.	II.2.146	27.	S2		

Green tea processing

Green tea was processed using a steaming method. A total of 500 g of manually harvested shoots consisting of a bud and three leaves (P+3) were steamed at approximately 200 °C under a pressure of 4 bar for 5 min. Following steaming, the shoots were air-dried for 1 h and subsequently pan-dried at 100 °C for 14 min. The leaves were then manually rolled for 15 min and finally dried in a tray dryer (14-mesh; 1.4 mm aperture size) at 100 °C for 30 min, following the procedure described by Prayoga *et al*. (2021).

Quality evaluation based on Indonesian National Standard (SNI)

The parameters evaluated according to the Indonesian National Standard (SNI) included moisture content, total ash content, crude fiber content, and polyphenol content. The analyses of moisture, total ash, and crude fiber contents were conducted at the Appropriate Technology Research Laboratories, National Research and Innovation Agency (BRIN), following the methods outlined in SNI 01-2891-1992. Meanwhile, the determination of polyphenol content was performed at the Plant Analysis Laboratory of the Research Institute for Tea and Cinchona, based on the method specified in ISO 14502-1:2005.

Protein levels

Protein levels was determined at the Appropriate Technology Research Laboratories, National Research and Innovation Agency (BRIN) using the Dumas combustion method with a DuMaster D-480 Protein Analyzer (BÜCHI Labortechnik AG, Flawil, Switzerland). Samples were combusted in an oxygen-rich atmosphere, and the released nitrogen was quantified using a thermal conductivity detector. Protein content was calculated from the total nitrogen concentration using an appropriate nitrogen-to-protein conversion factor (Buckee, 1994).

Cafein levels

The caffeine levels of the green tea samples was determined at the Plant Analysis Laboratory of the Research Institute for Tea and Cinchona using a standard gravimetric method, wherein the pulverized leaves were boiled in distilled water with sodium carbonate to precipitate tannins and extract the target compound (Nielsen, 2017). Following filtration, the aqueous extract was subjected to liquid-liquid extraction using dichloromethane to selectively partition the caffeine into the organic layer. The combined organic phases were then dehydrated with anhydrous sodium sulfate and evaporated to dryness under reduced pressure (AOAC International, 2005). Finally, the resulting crude caffeine residue was oven-dried to a constant weight, allowing the total caffeine content to be calculated as a mass percentage relative to the initial dry weight of the tea sample.

Statistical analysis

All chemical parameters tested are arranged in a Randomized Complete Block Design (RCBD) with two replications, where the 35 evaluated clones served as the main factor. The resulting data were subjected to an Analysis of Variance (ANOVA), followed by Tukey's Honestly Significant Difference (HSD) test at a 5% significance level. All statistical computations were performed using PKBT-Stat 3.2 software.

RESULTS AND DISCUSSION

The F-test results presented in Table 2 reveal varying degrees of significant differences in the biochemical composition among the 35 *Camellia sinensis* clones. Highly significant variations ($P < 0.01$) were observed in the contents of polyphenols, protein, and caffeine across the evaluated clones. Conversely, basic physicochemical parameters, including moisture, total ash, and crude fiber contents, showed no significant differences (ns).

The significant differences in polyphenols, protein, and caffeine contents are primarily driven by strong genetic control over secondary metabolite biosynthesis and nitrogenous compound accumulation in tea plants (Wang *et al*., 2022). Because these specific metabolites are the defining factors of tea quality, diverse genetic backgrounds among clones produce distinct chemotypic profiles, even when cultivated under identical environmental conditions (Bekele *et al*., 2022). In contrast, parameters such as moisture, total ash (mineral content), and crude fiber are fundamental physiological and structural components of the plant tissues. When grown under uniform agro-climatic conditions and subjected to the same plucking standards, these primary structural components remain highly stable and conserved across different clones of the same variety (Li *et al*., 2023).

Furthermore, the Coefficient of Variation (CV) for all evaluated biochemical parameters was relatively low, ranging from 3.32% for moisture to 9.38% for crude fiber. In agricultural and biological field trials, a CV value below 10% is considered excellent, indicating a high level of experimental precision, consistent sampling, and highly reliable analytical procedures (Kumar & Singh, 2024). This low variance relative to the mean confirms that the highly significant differences observed in the secondary metabolites are statistically robust, representing true genetic diversity among the clones rather than environmental or experimental noise.

Based on the biochemical evaluation of the 35 *Camellia sinensis* clones, there were no significant differences observed in the moisture and total ash contents. In contrast, highly significant variations were detected in the crude fiber, polyphenol, protein, and caffeine levels. The mean values for these significant parameters were further separated using Tukey's Honestly Significant Difference (HSD) test, demonstrating a wide and diverse range of biochemical profiles across the evaluated genotypes (Table 3).

Table 2. Anova Result

Chemical parameters	F_{Value}	F_{table}		CV (%)
		5%	1%	
Moisture content	0.82^{ns}	1.77	2.26	17.25
Total ash content	0.37^{ns}	1.77	2.26	12.02
Crude fiber content	4.65^{**}	1.77	2.26	4.45
Polyphenol content	10.02^{**}	1.77	2.26	2.67
Protein levels	15.05^{**}	1.77	2.26	6.48
Caffein levels	2.73^{**}	1.77	2.26	8.52

Explanation: * = significant at $P < 0.05$, ** = significant at $P < 0.01$, ns = not significant, CV = Coefficient of Variation

Each of these biochemical parameters plays a pivotal role in determining the final quality of green tea. Moisture and total ash contents are fundamental indicators of shelf-life stability and mineral purity, respectively. Crude fiber reflects the tenderness and maturity of the tea shoots, where lower levels are generally preferred to maintain physical quality. Polyphenols are the primary secondary metabolites responsible for the antioxidant capacity, astringency, and color of the tea liquor. Furthermore, protein content (along with its degraded amino acids) contributes significantly to the *umami* flavor and freshness, whereas caffeine imparts the characteristic bitterness and briskness to the infusion (Chen *et al*., 2022; Ye *et al*., 2023).

To ensure commercial viability, the evaluated parameters were compared against the Indonesian National Standard (SNI) for green tea. The minimal requirements and thresholds established by the SNI specify a maximum moisture content of 8%, a total ash content between 4% and 8%, a maximum crude fiber content of 16.5%, and a minimum polyphenol content of 11%. Based on the analytical results, all 35 evaluated clones successfully met these strict SNI requirements for moisture, total ash, crude fiber, and polyphenol contents, indicating their fundamental suitability for green tea production.

To further identify superior clones with optimal flavor profiles, the protein and caffeine levels were statistically categorized into classes based on their data distribution. The protein levels were divided into three classes using a minimum value of 22.26% and a maximum of 29.58%, yielding a range of 7.23. By specifying three classes, the class width was established at 2.44, resulting in the following categories: low (< 24.70%), medium (24.70% – 27.14%), and high (> 27.14%). Similarly, the caffeine levels were categorized using a minimum value of 3.0% and a maximum of 4.5% (range = 1.5). Applying a class width of 0.5, the caffeine content was classified as low (< 3.5%), medium (3.5% – 4.0%), and high (> 4.0%).

For premium-quality green tea, high protein content is considered desirable due to its contribution to a pronounced umami flavor, while low caffeine content is preferred to limit excessive bitterness (Liu *et al*., 2022). Considering these criteria together with compliance with the Indonesian National Standard (SNI), four superior clones were selected: I.1.70, I.2.45, I.2.85, and II.2.108. These clones satisfied the SNI quality requirements and were characterized by high protein contents (>27.14%) and low caffeine contents (<3.5%), highlighting their potential for the development and production of premium.

Table 3. Average chemical parameters content

Clones	Moisture Content (%)	Total ash content (%)	Crude fiber content (%)	Polyphenol content (%)	Protein levels (%)	Cafein levels (%)
I.1.100	**4.59^{a}**	**4.78^{a}**	**5.67^{cd}**	**15.70^{defgh}**	**27.70^{abcde}**	**3.41^{ab}**
I.1.101	**4.72^{a}**	**5.22^{a}**	**5.70^{cd}**	**17.10^{cdefg}**	24.49^{fghijk}	3.64^{ab}
I.1.70	**4.10^{a}**	**4.81^{a}**	**6.32^{abcd}**	**15.30^{efgh}**	26.88^{abcdefg}	**3.02^{b}**
I.1.93	**4.57^{a}**	**5.18^{a}**	**5.88^{abcd}**	**15.90^{defgh}**	**27.89^{abcd}**	4.53^{a}
I.2.188	**4.20^{a}**	**5.23^{a}**	**6.21^{abcd}**	**22.40^{ab}**	**28.49^{abc}**	3.93^{ab}
I.2.34	**5.13^{a}**	**5.06^{a}**	**6.65^{abcd}**	**18.80^{abcdef}**	22.91^{ijk}	**3.50^{ab}**
I.2.45	**4.22^{a}**	**5.14^{a}**	**5.84^{abcd}**	**16.40^{cdefg}**	**28.59^{ab}**	**3.50^{ab}**
I.2.85	**4.03^{a}**	**5.02^{a}**	**5.71^{cd}**	**20.40^{abcd}**	**29.58^{a}**	**3.09^{b}**
I.4.113	**3.74^{a}**	**5.03^{a}**	**5.67^{cd}**	**18.60^{abcdef}**	24.80^{efghijk}	3.62^{ab}
II.1.3	**4.19^{a}**	**5.57^{a}**	**5.78^{bcd}**	**21.10^{abc}**	22.26^{k}	**3.50^{ab}**
II.1.32	**4.06^{a}**	**5.16^{a}**	**6.08^{abcd}**	**18.40^{abcdef}**	25.80^{bcdefghi}	3.53^{ab}
II.1.38	**4.01^{a}**	**4.85^{a}**	**5.99^{abcd}**	**17.20^{cdefg}**	22.99^{ijk}	**3.28^{ab}**
II.1.60	**4.66^{a}**	**4.77^{a}**	**6.82^{abc}**	**22.90^{a}**	22.55^{jk}	3.51^{ab}
II.1.76	**4.43^{a}**	**5.00^{a}**	**5.83^{bcd}**	**20.20^{abcde}**	24.18^{ghijk}	3.54^{ab}
II.1.98	**4.49^{a}**	**4.60^{a}**	**6.57^{abcd}**	**17.90^{bcdefg}**	25.39^{defghij}	3.64^{ab}
II.2.108	**4.25^{a}**	**5.08^{a}**	**6.32^{abcd}**	**19.70^{abcde}**	**28.33^{abcd}**	**3.28^{ab}**
II.2.146	**4.14^{a}**	**5.10^{a}**	**6.18^{abcd}**	**18.10^{abcdef}**	23.48^{hijk}	**3.01^{b}**
II.2.43	**5.95^{a}**	**4.65^{a}**	**6.17^{abcd}**	**17.70^{bcdefg}**	25.51^{cdefghij}	**3.43^{ab}**
II.3.16	**3.63^{a}**	**4.69^{a}**	**6.73^{abcd}**	**15.90^{defgh}**	25.64^{bcdefghi}	3.89^{ab}
II.3.38	**4.07^{a}**	**4.52^{a}**	**5.91^{abcd}**	**20.00^{abcde}**	27.01^{abcdefg}	**3.12^{b}**
II.4.149	**3.96^{a}**	**4.69^{a}**	**5.81^{bcd}**	**19.00^{abcdef}**	26.14^{bcdefgh}	**3.32^{ab}**
II.4.178	**3.80^{a}**	**5.13^{a}**	**5.63^{d}**	**17.50^{bcdefg}**	**27.86^{abcd}**	3.51^{ab}
II.4.32	**4.31^{a}**	**4.52^{a}**	**6.18^{abcd}**	14.20^{fgh}	23.20^{hijk}	3.56^{ab}
R1	**4.02^{a}**	**4.93^{a}**	**6.04^{abcd}**	**20.20^{abcde}**	26.92^{abcdefg}	3.73^{ab}
R3	**4.26^{a}**	**4.93^{a}**	**5.64^{d}**	13.10^{gh}	**27.70^{abcde}**	4.18^{ab}
S1	**4.00^{a}**	**5.07^{a}**	**6.08^{abcd}**	**17.90^{bcdefg}**	25.59^{cdefghi}	3.79^{ab}
S2	**4.14^{a}**	**5.11^{a}**	**5.81^{bcd}**	**15.70^{defgh}**	25.60^{cdefghi}	**3.13^{b}**
S3	**4.04^{a}**	**4.96^{a}**	**6.51^{abcd}**	**16.70^{cdefg}**	26.98^{abcdefg}	**3.00^{b}**
SGMBA	**4.19^{a}**	**4.94^{a}**	**5.87^{abcd}**	**17.40^{cdefg}**	26.94^{abcdefg}	**3.21^{b}**
YABUKITA	**3.94^{a}**	**5.05^{a}**	**6.91^{ab}**	11.40^{h}	25.66^{bcdefghi}	**3.05^{b}**
GMBS 1	**4.09^{a}**	**5.41^{a}**	**6.58^{abcd}**	**17.60^{bcdefg}**	26.80^{abcdefg}	**3.25^{b}**
GMBS 2	**3.52^{a}**	**4.71^{a}**	**6.91^{ab}**	13.00^{gh}	**27.41^{abcdef}**	**3.43^{ab}**
GMBS 3	**4.83^{a}**	**4.77^{a}**	**6.24^{abcd}**	**18.30^{abcdef}**	26.77^{abcdefg}	3.61^{ab}
GMBS 4	**4.06^{a}**	**4.42^{a}**	**7.01^{a}**	**18.60^{abcdef}**	**27.94^{abcd}**	3.68^{ab}
GMBS 5	**3.39^{a}**	**4.96^{a}**	**6.65^{abcd}**	**21.10^{abc}**	**27.42^{abcdef}**	4.10^{ab}

Explanation: Numbers followed by the same letter in the same column are not significantly different based on the HSD test at the 5% level, numbers in bold correspond to the criteria.

Although clones I.1.70, I.2.45, I.2.85, and II.2.108 successfully met the SNI criteria and exhibited an optimal biochemical balance of high protein and low caffeine, a comprehensive quality assessment of green tea heavily relies on organoleptic evaluation. The total sensory value of the tea is determined by a cumulative scoring system encompassing five key parameters: dry appearance (scored 1–5), liquor color (1–5), taste (21–49), aroma (1–5), and infused leaf (1–5). Each parameter distinctly reflects specific aspects of the tea's quality and processing history.

The dry appearance indicates the plucking standard, mechanical integrity, and manufacturing precision (Zhu *et al*., 2021). Liquor color, which is driven by the preservation of chlorophyll and the prevention of polyphenol oxidation, serves as a primary indicator of freshness and proper fixation (*de-enzyming*) during processing. Taste, assigned the highest weight in the evaluation, represents the complex biochemical harmony between astringent catechins, bitter caffeine, and umami-rich amino acids derived from proteins. Furthermore, the aroma arises from volatile organic compounds that define the tea's characteristic fragrance, while the infused leaf reveals the degree of leaf tenderness and processing uniformity (Lin *et al*., 2022; Zhang *et al*., 2023).

Recent sensory profiling demonstrated that these four biochemically superior clones exhibited distinct organoleptic performances (Prayoga *et al*., 2025). Clones I.1.70 and I.2.45 obtained total sensory values of 78.83 and 75.33, respectively (Table 4). While these scores place them in the "good" quality category, they fall short of the highest commercial standard. In contrast, clones I.2.85 and II.2.108 achieved exceptional organoleptic scores, firmly classifying them into the "premium" quality category (Prayoga *et al*., 2025).

Table 4. Average value of green tea quality parameters clone I.2.45, I.2.85 and clone II.2.108

Clones	Dry appearance	Liquor color	Taste	Aroma	Infused leaf	Total value
I.1.70	4.00	3.50	40.00	4.00	4.17	78.83
I.2.45	3.73	3.07	39.33	3.67	4.13	75.33
I.2.85	3.93	3.67	41.00	4.43	4.20	81.07
II.2.108	3.93	4.03	43.67	4.17	4.33	84.57

Source: Prayoga et. al. (2025)

Ultimately, while biochemical profiling provides a foundational metric for potential quality, the true commercial value of green tea is dictated by human sensory perception. Because clones I.2.85 and II.2.108 uniquely integrate exceptional biochemical traits with premium organoleptic profiles, they represent the most superior genotypes among the evaluated population. Therefore, it is highly recommended that these two specific clones be prioritized for advanced agronomic propagation and large-scale commercial cultivation to support the production of premium-grade green tea.

CONCLUSION

In conclusion, this study highlights substantial biochemical and sensory diversity among the evaluated Indonesian sinensis tea clones. Clone I.2.85 and II.2.108 emerged as the most elite genotype for premium green tea production. It not only strictly fulfills all Indonesian National Standards (SNI) parameters—including optimal moisture, ash, crude fiber, and polyphenol contents—but also uniquely combines a high protein concentration with low caffeine levels, culminating in an exceptional flavor profile. Therefore, CloneI.2.85 and II.2.108 is highly recommended for commercial propagation to elevate the quality of the national green tea industry.

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