

Effect of Peptide Concentration On New Shoot Growth and Flowering of Arabica and Robusta Coffee

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ABSTRACT: Optimal new shoot formation and flowering stability are key to determining future coffee plant productivity, which is often hampered by unfavorable environmental conditions. This study aimed to identify differences in new shoot growth and generative components between Arabica and Robusta coffee plants, as well as to determine the most cost-effective peptide concentration. The study used a randomized block design (RBD) with seven replications, with coffee type as the main plot and peptide concentration as the subplot. There was no significant interaction between coffee type and peptide concentration for any parameter. As a single factor, coffee type significantly affected new shoot diameter and flower number per bunch, with Robusta coffee superior with a larger diameter (2.97 cm) and higher flower density (25.86 units) compared to Arabica (2.43 cm and 15.29 units) due to its high glomerular density. For nutrient factors, a concentration of 4 ml/L produced the maximum number of flowers per plant (8,981.04 units). However, in terms of cost efficiency, a concentration of 2 ml/L proved to be the best and most recommended choice. This dosage saved up to 50% on input costs while producing statistically equivalent results to the 4 ml/L dosage in terms of shoot length (4.21 cm), leaf number (2.38 units), shoot diameter (2.77 cm), and flower cluster number per plant (331.80 units). Overall, the 2 ml/L peptide concentration was highly effective for mass application.

KEYWORDS: Arabica coffee, robusta coffee, peptide, vegetative growth, new shoots.

I. INTRODUCTION

Coffee plants are a strategic plantation commodity with high economic value in both domestic and international markets. Throughout their life cycle, coffee plant productivity is largely determined by the balance and optimization of two main growth phases: the vegetative phase and the generative phase. Vegetative growth, characterized by the emergence of strong, long, leafy, and sturdy new shoots, plays a crucial role as the plant's primary architectural organ. These new shoots will later act as reproductive branches (sink-source translocation) and provide potential nodes (stem nodes) for the attachment of generative organs. Based on their inherent genetic traits, the two main commercial coffee varieties exhibit contrasting vegetative morphological variations; Robusta coffee genotypes naturally have a much stronger vascular tissue structure and denser mechanical support tissue compared to Arabica coffee plants (DaMatta *et al.*, 2022).

A successful and optimal vegetative phase will linearly stimulate the plant's readiness to enter the productive generative phase. The generative phase of the coffee plant is characterized by the formation of flower buds, which then develop into flower clusters. These inflorescence components, including the number of flowers per bunch, the number of bunches per branch, and the total number of flowers collected per plant, are early indicators of future coffee bean yield capacity and potential. Regarding the botanical structure of these inflorescences, Robusta coffee is characterized by producing flowers in whorls at each stem node, known as glomerular density. Its internode capacity is genetically designed to be much denser and more compact than Arabica coffee (Teixeira *et al.*, 2022; Ramalho *et al.*, 2023). However, in reality, this phase transition is often hampered by limited soil nutrient uptake, microclimate fluctuations, and a high rate of potential flower abortion (dropping) before developing into ripe fruit.

One recent effort to optimize these two growth phases is through the use of short-chain peptide (oligopeptide)-based biostimulants. These active peptide compounds function as signaling molecules capable of binding to kinase receptors on the cell membrane to accelerate cell division in the apical meristem (Albert *et al.*, 2022). In addition to stimulating vegetative bud formation, peptides act as biological elicitors, modifying the balance of endogenous hormones (such as cytokinins and

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gibberellins) to stimulate the simultaneous expression of flowering initiation genes (Franzoni *et al.*, 2023). Through peptide application, nitrogen metabolism, nutrient uptake efficiency, and the activity of photosynthetic leaf carboxylase enzymes can be enhanced (Rouphael *et al.*, 2023; Carillo *et al.*, 2024). This readily available amino acid uptake bypasses the conventional nitrogen assimilation pathway, enabling a crucial energy-saving mechanism for smoothly transporting carbohydrates to reproductive organs while minimizing the risk of flower drop under fluctuating environmental conditions (Colla *et al.*, 2024).

Therefore, considering the crucial relationship between the vegetative structure of new buds and the success of the generative components of inflorescences, this study was conducted to comprehensively examine the interaction and single effects of coffee type and peptide biostimulant concentration on both growth phases.

II. RESEARCH METHODOLOGY

Research Location and Time

This research was conducted from October 2024 to October 2025. Field activities were located in Tambakan Village, Kubutambahan District, Buleleng Regency. Geographically, the research area is located in the Arabica and Robusta coffee cultivation zone with a topography altitude ranging between 1000–1200 meters above sea level (masl) and slope characteristics dominated by a slope class of 25–45% (steep category).

Materials and Equipment

The main materials used were 42 coffee trees, consisting of Arabica (*Coffea arabica*) and Robusta (*Coffea canephora*) coffee, as well as a peptide solution as a biostimulant treatment. The tools used included tape, a ruler, calipers, writing instruments, and other cultivation support tools.

Experimental Design

This research was conducted using a Factorial Randomized Block Design (RBD) with a Split Plot Design pattern consisting of 2 factors and 7 replications.

Main Plot: Coffee Type (K)

- Ka = Arabica Coffee
- Kr = Robusta Coffee

Subplot: Peptide Concentration (P)

- P0 = Peptide concentration 0 ml/L water (control)
- P2 = Peptide concentration 2 ml/L water
- P4 = Peptide concentration 4 ml/L water

The combination of these two factors resulted in 6 treatments replicated 7 times, resulting in a total of 42 experimental units (coffee trees) with the following combination details:

- KaP0: Arabica Coffee + peptide 0 ml/L water
- KaP2: Arabica Coffee + peptide 2 ml/L water
- KaP4: Arabica Coffee + peptide 4 ml/L water
- KrP0: Robusta Coffee + peptide 0 ml/L water
- KrP2: Robusta Coffee + peptide 2 ml/L water
- KrP4: Robusta Coffee + peptide 4 ml/L water

Research Implementation

This study began with the selection of 6–8-year-old Arabica and Robusta coffee sample trees (full production period) that were homogeneous in terms of height, crown size, and plantation management history in Tambakan Village. Plant maintenance was carried out in accordance with good coffee cultivation SOPs, including routine weeding, fertilization using manure, integrated pest and disease management (IPM), and pruning to stimulate new shoot growth. Peptide treatment (product of CH. Biotech Co. Ltd, Taiwan) was applied by spraying the crown at concentrations of 0 ml/L (control), 2 ml/L, and 4 ml/L of water using a low-pressure sprayer. The peptide solution was applied four times during the growth cycle: 15 days before flowering, during fruit set, 1.5 months after fruit set to support fruit development, and when the fruit began to ripen (turn red).

Observation Variables

The variables observed included parameters of vegetative, namely:

1. Length of new shoots (cm)

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2. Number of leaves on new shoots (cm)
3. Diameter of new shoots (cm)
4. Number of flowers per cluster (unit)
5. Number of flower clusters per branch (unit)
6. Number of flower clusters per plant (unit)
7. Number of flowers per plant (flowers)

Vegetative variables included new shoot length, shoot diameter (± 1 cm from the base), and number of leaves, averaged from 5 random shoots per tree. Generative variables consisted of the number of flowers per bunch (averaged from 10 random bunches) and flower bunches per branch (averaged from 5 branches). In addition, the total number of bunches per plant was calculated by multiplying the bunches per branch by the total number of tree branches, while the total number of flowers per plant was obtained by multiplying the flowers per bunch by the total number of bunches per plant.

Data Analysis

Observational data were analyzed using analysis of variance (ANOVA) or variance (F test) at a 5% significance level to test the effect of the treatment on the observed variables. If the analysis of variance results indicate that one factor has a significant effect, further testing is performed using the Least Significant Difference (LSD) test to determine differences between treatments. If the interaction or combination of factors shows a significant effect, further testing is performed using the 5% Duncan test to determine which treatment groups are significantly different.

III. RESULTS

The interaction between coffee type and peptide application did not show a significant effect. As a single factor, coffee type only had a very significant effect on the diameter of new shoots and the number of flowers per bunch, while for the length of new shoots, the number of leaves of new shoots, the number of flower clusters (per branch and per plant), and the total number of flowers per plant, the effects were not statistically significant. Meanwhile, the single factor of peptide application had a very significant effect on the diameter of new shoots and all flower components (number of flowers per bunch, number of flower clusters per branch, number of flower clusters per plant, and number of flowers per plant), and had a significant effect on the parameters of new shoot length and number of leaves of new shoots.

Table 1. Significance of the Effect of Peptide Concentration on Vegetative Growth of Arabica and Robusta Coffee

No	Variables	Treatment		
		K	P	K x P
1	Length of new shoots	ns	*	ns
2	Number of leaves on new shoots	ns	*	ns
3	Diameter of new shoots	**	**	ns
4	Number of flowers per cluster	**	**	ns
5	Number of flowers cluster per branch	ns	**	ns
6	Number of flowers cluster per plant	ns	**	ns
7	Number of flowers per plant	ns	**	ns

Note: ns = unreal ($P \geq 0.05$); * = real ($P < 0.05$); ** = very real ($P < 0.01$).

Peptide application at a concentration of 4 ml/L resulted in the longest new shoots (4.43 cm), an increase of 15.06% and significantly different from the control (3.85 cm), but statistically equivalent to the concentration of 2 ml/L (4.21 cm). In contrast, the coffee type factor did not have a significant effect, although Robusta coffee showed a slightly longer growth trend (4.35 cm) compared to Arabica coffee (3.99 cm).

Table 2. Effect of Peptide Concentration on Vegetative Growth in Arabica and Robusta Coffee Plants

Treatment	Length of new shoots (cm)	Number of leaves on new shoots (unit)	Diameter of new shoots (cm)
Types of Coffee (K)			
K _a	3,99 a	2,35 a	2,43 b
K _r	4,35 a	2,37 a	2,97 a
BNT 5%	0,37	0,13	0,17

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Peptide Concentration (P)			
P ₀	3,85 b	2,25 b	2,51 b
P ₂	4,21 a	2,38 ab	2,77 a
P ₄	4,43 a	2,46 a	2,81 a
BNT 5%	0,29	0,16	0,20

Note: The number followed by the same letter in the same column shows no significant difference in the BNT test at 5%.

The highest number of leaves was obtained with 4 ml/L peptide treatment (2.46 units), increasing by 9.33% and significantly different from the control (2.25 units), while the 2 ml/L concentration (2.38 units) gave results equivalent to the 4 ml/L treatment. Meanwhile, the coffee type factor showed a uniform growth response and did not differ significantly between Robusta coffee (2.37 units) and Arabica coffee (2.35 units).

The 4 ml/L peptide treatment produced the largest shoot diameter (2.81 cm), an increase of 11.95% from the control (2.51 cm) and no significant difference with the 2 ml/L concentration (2.77 cm). In contrast to the length and number of leaves, the coffee type factor had a significant single effect, where Robusta coffee produced a significantly thicker shoot diameter (2.97 cm) compared to Arabica coffee (2.43 cm).

Table 3. Effect of Peptide Concentration on Generative Growth in Arabica and Robusta Coffee Plants

Treatment	Number of flowers per bunch (unit)	Number of flower clusters per branch (unit)	Number of flower clusters per plant (unit)	Number of flowers per plant (flower)
Types of Coffee (K)				
K _a	15,29 b	5,98 a	287,98 a	4.952,55 a
K _r	25,86 a	5,56 a	297,92 a	7.953,13 a
BNT 5%	3,37	0,61	128,98	3.165,49
Peptide Concentration (P)				
P ₀	16,37 c	5,08 c	181,21 b	3.085,68 c
P ₂	20,96 b	5,74 b	331,80 a	7.291,79 b
P ₄	24,40 a	6,49 a	365,84 a	8.981,04 a
BNT 5%	1,66	0,58	64,37	1.530,11

Note: The number followed by the same letter in the same column shows no significant difference in the BNT test at 5%.

The 4 ml/L peptide treatment produced the highest number of flowers per cluster (24.40 units), which significantly differed from the 2 ml/L concentration (20.96 units) and the control (16.37 units). Coffee species also had a significant effect, with Robusta yielding significantly more flowers (25.86 units) than Arabica (15.29 units). Peptide application significantly affected the number of clusters per branch, peaking at 4 ml/L (6.49 units), followed by 2 ml/L (5.74 units), and lowest in the control (5.08 units). Conversely, coffee species exerted no significant influence, although Arabica trended slightly higher (5.98 units) than Robusta (5.56 units).

The highest number of flower clusters per plant was recorded at 4 ml/L peptide (365.84 units), which was statistically equivalent to the 2 ml/L dose (331.80 units), but both were significantly higher than the control (181.21 units). Meanwhile, coffee species showed no significant difference. The 4 ml/L peptide concentration achieved the highest number of flowers per plant (8,981.04 flowers), significantly outperforming the 2 ml/L concentration (7,291.79 flowers) and the control (3,085.68 flowers). The coffee species factor was statistically non-significant, despite Robusta recording a higher average value (7,953.13 flowers) than Arabica (4,952.55 flowers).

IV. DISCUSSION

Peptide biostimulant application had significant to highly significant effects on improving all vegetative growth parameters of new coffee shoots. For new shoot length, the 4 ml/L (P₄) treatment resulted in the best linear growth, averaging 4.43 cm, representing a significant increase of 15.06% compared to the control treatment (P₀), which only produced a shoot length of 3.85 cm. Although the P₄ treatment was not statistically significantly different from the 2 ml/L (P₂) treatment, which achieved 4.21 cm, both doses significantly outperformed the control. A similar pattern was observed for the number of new shoot leaves, with the P₄ concentration triggering the highest leaf formation (2.46 units), significantly different from the control (2.25 units) but at a similar level of homogeneity to the P₂ treatment (2.38 units). On the other hand, the single factor of coffee variety showed a

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uniform or insignificant response to shoot length and leaf number, suggesting that the capacity for lateral organ initiation in this early phase is largely driven by exogenous nutrient stimulation.

A different situation was found for the new shoot diameter parameter, where, in addition to being significantly influenced by peptide concentration, this parameter was also significantly influenced by the single factor of coffee variety. Coffee plants treated with 4 ml/L peptide (P4) produced the largest shoot diameter at 2.81 cm, followed by P2 at 2.77 cm, both significantly thicker than the control, which only reached 2.51 cm. In terms of genotype, Robusta coffee consistently produced significantly thicker and sturdier new shoot diameters, reaching 2.97 cm, significantly different from Arabica coffee, which only had an average shoot diameter of 2.43 cm.

Physiologically, the success of peptide application in improving vegetative performance is closely related to its role as a signaling molecule. Short-chain peptides (oligopeptides) applied through the leaf canopy are readily absorbed through stomata and then bind to kinase receptors on the cell membrane to activate endogenous growth hormones such as auxins and brassinosteroids (Albert *et al.*, 2022). This activation accelerates cell division and elongation in the apical meristem tissue. Furthermore, peptide biostimulants can regulate the transcripts of transporter genes in roots to increase the efficiency of nutrient uptake (nitrogen and iron) and optimize the activity of photosynthetic enzymes (Rouphael *et al.*, 2023). The uptake of readily available amino acids bypasses the conventional, energy-intensive nitrogen assimilation pathway, resulting in an energy-saving mechanism. This saving is particularly important for plants growing in areas with steep topography and fluctuating climates, such as in Tambakan Village, as the remaining energy can be allocated to strengthening the growth of lateral shoot structures (Colla *et al.*, 2024). On the other hand, the larger shoot diameter in Robusta coffee is driven by an innate genetic trait. Anatomically, Robusta coffee genotypes have a vascular tissue structure (xylem-phloem) and a density of supporting mechanical tissue (sclerenchyma) that is naturally much more massive than Arabica coffee (DaMatta *et al.*, 2022).

The strong synergy between the provision of organic nutrients in the form of peptides and the expression of plant genetic potential is clearly visible in all components of the coffee plant inflorescence presented in Table 5.3. In the parameter of the number of flowers per bunch, the 4 ml/L concentration treatment (P4) provided the most optimal results with an average of 24.40 flower units, significantly different from the P2 concentration (20.96 units) and the P0 control (16.37 units). In addition to nutritional factors, the single factor of coffee type also had a very significant influence, where Robusta coffee produced a much higher flower density (25.86 units per bunch) compared to Arabica coffee which only produced 15.29 units per bunch. The linear effect of peptide application was again shown in the parameters of the number of flower clusters per branch and per plant. The administration of the P4 peptide concentration triggered the highest number of flower clusters per branch at 6.49 units, followed by P2 at 5.74 units, and the P0 control at 5.08 units. When these parameters were converted to a macroscale per plant, the P4 treatment produced the highest total flower clusters (365.84 units), significantly higher than the control (181.21 units) but statistically equivalent to the P2 treatment, which achieved 331.80 flower clusters per plant.

The culmination of all these inflorescence components culminated in the total number of flowers per plant, which showed a significant increase due to the peptide intervention. Coffee trees treated with 4 ml/L peptide (P4) produced an impressive total of 8,981.04 flowers per plant, followed by the P2 treatment with 7,291.79, and the lowest yield was achieved by the P0 control with only 3,085.68. The high-dose peptide application (P4) successfully increased the flowering capacity of the plants by up to 191% compared to the untreated condition. In contrast, coffee varieties showed no statistically significant effect on either the number of flower clusters or the total number of flowers per plant. This lack of significant differences is due to the high natural variability between plant blocks in the highlands of the study site, although Robusta coffee showed significantly higher accumulation levels in absolute data.

The mechanism behind the peptide's ability to enhance generative components is related to its role as a biological elicitor that modifies the balance of endogenous hormones, particularly the cytokinin and gibberellin groups, and stimulates the expression of target genes encoding flowering initiation (Franzoni *et al.*, 2023). The availability of functional amino acids from the peptide at a critical plant stage (15 days before flower formation) helps optimize the carbon-to-nitrogen (C/N) ratio in the leaf mesophyll tissue, thereby increasing chlorophyll and photosynthetic efficiency (Carillo *et al.*, 2024). This smooth flow of carbohydrates (source-to-sink translocation) to flower primordia successfully minimizes nutrient competition between flower primordia, thereby reducing the rate of abortion, or the loss of flower primordia before full bloom. Regarding Robusta coffee's absolute superiority in flower density per bunch, this is a taxonomic characteristic of the plant. Robusta coffee is characterized by overlapping, whorled inflorescences at each stem node, known as glomerular density. The capacity and differentiation of primordia in its nodes are genetically engineered to be much denser and more compact than Arabica coffee (Teixeira *et al.*, 2022; Ramalho *et al.*, 2023).

V. CONCLUSION

This study showed no significant interaction between coffee type and peptide concentration on vegetative growth parameters of new shoots or inflorescence components of coffee plants. However, as a single factor, there were significant genetic differences in growth, with Robusta coffee superior in producing thicker new shoot diameters (2.97 cm) and higher flower density per bunch (25.86 units) compared to Arabica coffee. Meanwhile, the best peptide concentration recommended statistically and economically was 2 ml/L. This dosage was significantly more efficient, saving farmers up to 50% in input costs while still providing equivalent (not significantly different) yields to the maximum dosage of 4 ml/L for vegetative parameters and the number of flower bunches per plant.

Based on these results, farmers are advised to use peptide biostimulants at a concentration of 2 ml/L for mass application in the field to optimize financial returns without sacrificing plant growth quality. The 4 ml/L dosage is only recommended as an alternative if farmers have sufficient capital and want to achieve the maximum total flower number target, particularly for Robusta coffee. Furthermore, further research is recommended to analyze the actual agricultural profit/loss ratio (R/C ratio) from the time the crop enters the fruit filling phase until harvest.

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