International Journal of Agricultural Technology 2026 Vol. 22(4):1649-1668
DOI: https://doi.org/10.63369/ijat.2026.22.4.1649-1668
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Effects of season and substrate on cutting propagation efficiency and nursery growth of selected ancient Shan tea genotypes (*Camellia sinensis* var. *assamica*) in Northern Vietnam

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Bui, T. L., Tran, T. P. L., Le, C. T., Nguyen, V. T., Ong, X. P., Chu, D. H., La, V. H., Soytong, K. and Song, J. J. (2026). Effects of season and substrate on cutting propagation efficiency and nursery growth of selected ancient Shan tea genotypes (*Camellia sinensis* var. *assamica*) in Northern Vietnam. International Journal of Agricultural Technology 22(4):1649-1668.

Abstract The effects of seasonal variations and different types of substrates on the survival rate, rooting ability, and growth of Shan tea plants (*Camellia sinensis* var. *assamica*) during the seedling stage using cuttings are investigated. The selected ancient Shan tea trees is developed the new tea varieties, with cuttings taken in two seasons (summer-autumn and winter-spring) and three types of substrates (including yellow-red ferralitic soil, sand, and rice husk ash with different mixing ratios). Evaluation based on indicators such as survival rate, rooting rate, and growth characteristics of roots, stems, and leaves showed that seedlings from different lines exhibited varying growth patterns, with lines LC.TT16 and YB.SB4 showed the best growth performance in both winter-spring and summer-autumn seasons. The substrates are supplemented with sand and rice husk ash which is helped the plants develop better root systems and shoot growth as compared to plain red-yellow ferralitic soil, highlighting the important role of porosity and suitable moisture retention of the substrate. Vegetative propagation of Shan tea through cuttings are improved the quality of seedlings, contributing to the conservation and sustainable development of Shan tea genetic resources.

Keywords: Cutting propagation, Growth, Propagation season, Shan tea, Substrate

Introduction

Tea (*Camellia sinensis* (L.) O. Kuntze) is one of the most important long-standing industrial crops, and its products are used by billions of consumers as the most popular non-alcoholic beverage worldwide (Chen *et al*., 2012; FAOSTAT, 2023; Wang *et al*., 2020). Among tea varieties, *Camellia sinensis* var.

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assamica is characterized by large leaves, vigorous growth, and rich phytochemical components, making it an important genetic source for tea production and propagation in Asia (Liu *et al*., 2017; Phong *et al*., 2016; Xia *et al*., 2017).

In Vietnam, Shan tea (*Camellia sinensis* var. *assamica*), locally known as "Shan Tuyet," is mainly distributed in high mountainous areas of northern provinces such as Son La, Lao Cai, Dien Bien, and Tuyen Quang... These tea populations mainly consist of ancient and semi-wild tea trees adapted to harsh ecological conditions over a long period and are considered valuable indigenous genetic resources (Nguyen *et al*., 2022). Old-growth Shan tea trees (*Camellia sinensis* var. *assamica*) are rich in secondary metabolites, especially polyphenols, catechins, caffeine, and free amino acids, which contribute to the biological activity and taste of tea. Recent studies have shown that catechins and flavonoid derivatives in tea have strong antioxidant capabilities, contributing to cardiovascular protection, metabolic regulation, and supporting the prevention of certain chronic diseases (Kochman *et al*., 2020; Kottawa-Arachchi *et al*., 2019). In addition, the harsh environmental conditions at high altitudes also promote tea plants to accumulate phenolic compounds as a physiological adaptation mechanism, which in turn enhances the flavor and potential medicinal value of tea leaves (Ahmed and Stepp, 2025). Therefore, ancient Shan tea populations not only have economic value but also serve as a precious gene source for breeding and developing high-quality tea products.

Vegetative propagation plays an important role in the utilization and conservation of ancient tea gene varieties. In commercial tea production, propagation by cuttings is the main method because it preserves desirable genetic traits, ensures uniformity of tea gardens, yields high productivity, and shortens the seedling period compared to seed propagation (Ahmad *et al*., 2015; Choubey *et al.*, 2013). However, the success of cutting propagation depends on many factors, including the gene type, physiological state of the mother plant, cutting position, season, substrate, and environmental conditions during rooting (Shrestha and Miles, 2025; Zhou *et al*., 2025a), as well as some diseases that harm tea roots (La *et al*., 2016). Therefore, the aim of this study was to evaluate the effects of season and substrate on the efficiency of cutting propagation and the growth of tea seedlings in nurseries of selected Shan tea (*Camellia sinensis* var. *assamica*) lines from the Northern Vietnam, providing a scientific basis for the selection, conservation, and sustainable development of Shan tea gene resources.

Materials and methods

Plant materials and study sites

Five ancient Shan tea trees (*Camellia sinensis* var. *assamica*) were used in the study. These genotypes had previously been evaluated based on morphological traits and selected as elite lines. The materials included HG.LT1 collected in Cao Bo commune, Tuyen Quang province; YB.SB4 collected in Van Chan commune, Lao Cai province; and three lines LC.TT5, LC.TT11, and LC.TT16 collected in Cao Son commune, Lao Cai province. The sampling locations are in the high mountainous region of northern Vietnam, at altitudes of 912–950 m, 737–768 m, and 1128–1172 m above sea level, respectively. The mother plants were selected for being healthy, stably growing, and free of pests and diseases at the time of sampling. Cuttings were collected and propagated in two different seasons, corresponding to summer (April 2024) and winter (November 2024). The nursery is located at Ban Xen tea Cooperative, Ban Lau commune, Lao Cai province, Vietnam.

Cutting preparation and propagation technique

Vegetative propagation was carried out by cutting methods according to previously published tea propagation protocols, with adjustments suitable for local conditions (Ahmad *et al.*, 2015; Do *et al.*, 2010). The cuttings were taken from semi-mature (semi-woody) branches, free of pests and diseases. The cuttings consist of one leaf and one node, measuring 3-5 cm in length. For larger leaves, a portion of the leaf surface is cut off to reduce water loss during the initial rooting stage. The base of the cutting is beveled at an angle of approximately 45° to increase the contact surface and facilitate adventitious root formation. After preparation, the cuttings are directly inserted into the substrate in the nursery and cared for under controlled environmental conditions.

Nursery conditions

The tea cuttings were used in a nursery with a covered shelter, where environmental conditions were maintained relatively stable to facilitate callus formation and adventitious root development. Air humidity, temperature, and shading were adjusted appropriately to meet the physiological requirements of the cuttings during the rooting stage. The substrate is regularly watered to maintain the necessary moisture while ensuring good drainage and aeration.

Experimental design for evaluating the effect of propagation season

The experiment evaluating the effect of the propagation season on vegetative propagation ability of selected Shan tea lines was completely randomized with two propagation seasons: summer–autumn (seedling in April) and winter–spring (seedling in November). Five outstanding Shan tea varieties (HG.LT1, YB.SB4, LC.TT5, LC.TT11, and LC.TT16) were propagated by cuttings under the same nursery conditions for each season. Each treatment was arranged with 3 replicates, each consisting of 50 cuttings. The duration from planting to evaluation was 10 months.

Experimental design for evaluating the effect of substrate

The experiment assessing the effect of the substrate on the survival rate, rooting, and growth of tea cuttings was conducted on the selected Shan tea line LC.TT16. LC.TT16 was chosen because of its outstanding propagation ability in previous experiments. Three substrate formulas were used, including CT1 (pure yellow–red ferralitic soil), CT2 (10% sand + yellow-red ferralitic soil), and CT3 (5% sand + 10% rice husk ash + yellow-red ferralitic soil); clean soil, free of impurities, with a pH of 4.5–5.0. The experiment was completely randomized, with each treatment replicated three times, each replicate consisting of 50 cuttings. All treatments were maintained under the same care conditions in the same nursery.

The evaluated parameters included survival rate, callus formation rate, root emergence rate, and bud emergence rate, which were assessed after 2 months; survival rate and transplantable seedling rate were determined after 10 months. Morphological growth and biomass of the seedlings were monitored at 6 and 10 months after planting using standard measurement methods commonly applied in plant physiology and propagation studies.

Statistical analysis

Data were firstly tested for normality and homogeneity of variance prior to statistical analysis. Analysis of variance (ANOVA) was performed to determine significant differences among treatments. Mean comparisons were conducted using an appropriate post-hoc test at a significance level of $p < 0.05$. All values are presented as mean ± standard deviation (SD).

Results

Effect of propagation season on survival, rooting, and nursery performance of Shan tea cuttings

Root formation and bud emergence in tea cuttings propagation are important processes influenced by both genotype and environmental conditions. In this study, were propagated by cuttings in April 2024 (summer-autumn season) and November 2024 (winter-spring season). The effect of the summer-autumn season on survival rate, rooting ability, and nursery performance of cuttings from five selected shan tea lines are presented in Table 1 and Table 2.

Table 1. Effect of the summer-autumn season on survival rate, rooting ability, and nursery performance of cuttings from five selected shan tea lines

Parameters / Genotype	After 2 months				After 10 months	
	Survival rate (%)	Callus formation rate (%)	Rooting rate (%)	Bud emergence rate (%)	Survival rate (%)	Transplantable seedling rate (%)[1]
LC.TT5	81.33±1.16[b/2]	78.67±2.31[c]	72.00±2.00[b]	73.33±3.06[b]	70.00±2.00[b]	58.67±1.16[b]
LC.TT11	83.33±3.06[ab]	82.00±2.00[ab]	74.00±2.00[ab]	76.00±2.00[ab]	72.00±2.00[ab]	60.00±2.00[ab]
LC.TT16	85.33±1.16[a]	83.33±1.16[a]	75.33±1.16[a]	78.00±2.00[a]	74.00±2.00[a]	62.67±2.31[a]
YB.SB4	84.67±1.16[ab]	81.33±1.16[abc]	75.33±2.31[a]	77.33±3.06[ab]	72.67±1.16[ab]	62.67±1.16[a]
HG.LT1	84.00±2.00[ab]	79.33±1.16[bc]	73.33±1.16[ab]	76.00±2.00[ab]	71.33±1.16[ab]	61.33±1.16[ab]
CV %	3.81	4.38	3.07	5.59	3.81	4.88
LSD 0,05	0.060	0.067	0.042	0.080	0.052	0.056

[1/] Seedlings were considered suitable for nursery release when they reached a height ≥ 25 cm, basal stem diameter ≥ 3 mm, possessed 8–10 true leaves, and exhibited strong, healthy growth.
[2/] Different lowercase letters indicate statistically significant differences among the studied lines within the same column ($p < 0.05$).

Table 2. Effect of the winter–spring season on survival, rooting ability, and nursery performance of cuttings from five selected shan tea lines

Parameters / Genotype	After 2 months				After 10 months	
	Survival rate (%)	Callus formation rate (%)	Rooting rate (%)	Bud emergence rate (%)	Survival rate (%)	Transplantable seedling rate (%)
LC.TT5	87.33±3.06[c/1]	82.00±2.00[b]	74.67±3.06[b]	76.67±2.31[c]	70.67±2.00[b]	61.33±3.06[c]
LC.TT11	92.67±1.16[ab]	86.67±4.16[ab]	77.33±3.06[ab]	79.33±2.31[abc]	73.33±3.06[ab]	62.67±1.16[bc]
LC.TT16	93.33±1.16[a]	88.00±3.46[a]	80.67±2.31[a]	83.33±3.06[a]	76.00±2.00[a]	67.33±1.16[a]
YB.SB4	92.00±2.00[ab]	85.33±3.06[ab]	79.33±1.16[a]	82.67±3.06[ab]	75.33±2.31[a]	66.67±2.31[ab]
HG.LT1	90.00±2.31[bc]	83.33±3.06[ab]	76.00±2.00[ab]	78.00±2.00[bc]	73.33±3.06[ab]	63.33±2.00[abc]
CV%	1.75	3.42	4.10	3.56	2.87	4.00
LSD 0,5	0.030	0.055	0.060	0.054	0.040	0.048

[1/] Different lowercase letters indicate statistically significant differences among the studied lines within the same column ($p < 0.05$).

The results indicated that during the summer-autumn season, the selected Shan tea lines exhibited high vegetative propagation performance (Table 1). The initial survival rate ranged from 81.33% to 85.33%, with the LC.TT16 clone having the highest (85.33%) and the LC.TT5 clone the lowest (81.33%). After the callus formation stage (78.67%–83.33%), the rooting rate reached 72.00%–75.33%; the LC.TT16 and YB.SB4 clones (75.33%) outperformed the LC.TT5 clone (72.00%). A similar trend was observed in subsequent indicators:- bud emergence rate of 73.33%–78.00%, survival rate after 10 months of 70.00%–74.00%, and the transplantable seedling rate ranged from 58.67% to 62.67%.

In addition to seasonal factors, the influence of genetic type was also evident with the LC.TT16 and YB.SB4 clones showing high callus formation rates (83.33% and 81.33%). The LC.TT16 also had the highest rooting, 10-month survival, and transplantable seedling rate (74.00% and 62.67%), while the LC.TT5 clone had the lowest in most indicators.

Result in winter-spring season showed that all selected Shan tea lines exhibited high vegetative propagation capacity by stem cuttings, with differences among genotypes (Table 2). The initial survival rate ranged from 87.33% (LC.TT5 clone) to 93.33% (LC.TT16 clone). The callus formation rate ranged from 82.00% to 88.00%, depending on the tea clones, with root emergence rates reaching 74.67–80.67%, notably higher in LC.TT16 (80.67%) and YB.SB4 (79.33%) compared to LC.TT5 (74.67%). This trend continued in subsequent stages, with bud emergence rate from 76.67–83.33%, survival after 10 months from 70.67–76.00%, and transplantable seedling rate from 61.33–67.33%. Although the root emergence rate is high, the transplantable seedling rate was 61.33–67.33%. The results were due to the fact that the plants within the population had different growth rates. In reality, the remaining plants that do not meet the export standards would continue to be cared for and export once they meet the required standards. The differences between the lines are reflected the dominant role of genetic factors in the formation of adventitious roots. The LC.TT16 and YB.SB4 lines had high callus formation rates (88.00% and 85.33%) and survival rates after 10 months (76.00% and 75.33%), whereas LC.TT5 consistently showed the lowest values across these parameters.

Effect of propagation season on the growth of Shan tea cuttings in the nursery stage

In both the summer-autumn and winter-spring seasons, the growth characteristics of Shan tea were evaluated at 2, 6, and 10 months after cutting propagation.

At the 2-month stage, the cuttings adapted to the substrate environment and formed callus, with some lines beginning to root but with few short roots (0-2 roots), and no bud emergence was observed (Figure 1). Therefore, growth indicators such as shoot length, shoot biomass, leaf characteristics, and seedling stem diameter were measured at 6 and 10 months, corresponding to Tables 3 and 4 (summer-autumn season), and Tables 5 and 6 (winter-spring season), respectively.

Figure 1. (A, B) Cutting preparation and (C) cuttings at 2 months after propagation

Table 3. Growth performance of selected Shan tea lines under nursery conditions at 6 months after propagation in the summer–autumn season

Parameters / Genotype	Shoot length (cm)	Seedling stem diameter (mm)	Fresh root weight (g)	Shoot biomass (g)	Leaf weight (g)	Number of primary roots
LC.TT5	5.54±0.52$^{ab/1}$	1.56±0.21^{b}	0.20±0.04^{b}	0.58±0.07^{ab}	0.28±0.02^{bc}	3.20±0.45^{ab}
LC.TT11	5.10±0.83^{ab}	1.54±0.19^{b}	0.15±0.03^{b}	0.52±0.08^{bc}	0.29±0.03^{ab}	3.40±0.55^{ab}
LC.TT16	6.08±0.80^{a}	1.72±0.15^{ab}	0.20±0.06^{b}	0.66±0.08^{a}	0.33±0.03^{a}	3.60±0.55^{ab}
YB.SB4	5.64±1.08^{ab}	1.92±0.19^{a}	0.34±0.08^{a}	0.53±0.07^{bc}	0.29±0.03^{b}	3.80±0.84^{a}
HG.LT1	4.74±0.91^{b}	1.64±0.25^{b}	0.14±0.03^{b}	0.49±0.03^{c}	0.24±0.04^{c}	2.80±0.37^{b}
CV %	14.03	12.68	25.53	12.61	10.91	19.74
$LSD_{0,05}$	1.02	0.28	0.07	0.09	0.42	0.89

[1/] Different lowercase letters indicate statistically significant differences among the studied lines within the same column ($p < 0.05$).

Table 4. Growth performance of selected Shan tea lines under nursery conditions at 10 months after propagation in the summer–autumn season

Parameters / Genotype	**Shoot length (cm)**	**Seedling stem diameter (mm)**	**Fresh root weight (g)**	**Shoot biomass (g)**	**Leaf weight (g)**	**Number of primary roots**
LC.TT5	32.70±2.86$^{b/1}$	3.36±0.21^{b}	1.18±0.03^{b}	4.69±0.51^{b}	2.33±0.30^{b}	6.17±1.67^{a}
LC.TT11	28.80±1.74^{c}	3.32±0.30^{b}	1.21±0.15^{b}	4.53±0.55^{b}	2.46±0.52^{b}	5.67±0.82^{a}
LC.TT16	37.44±2.15^{a}	3.68±0.18^{a}	1.64±0.22^{a}	5.56±0.29^{a}	3.60±0.39^{a}	6.33±1.03^{a}
YB.SB4	36.98±2.29^{a}	3.68±0.15^{a}	1.33±0.13^{b}	5.60±0.32^{a}	3.10±0.41^{a}	5.67±1.03^{a}
HG.LT1	33.72±1.99^{b}	3.58±0.19^{ab}	1.37±0.25^{b}	5.96±0.48^{a}	3.25±0.40^{a}	6.17±0.75^{a}
CV %	5.59	6.10	14.10	8.21	13.95	17.08
$LSD_{0,05}$	2.54	0.29	0.25	0.58	0.55	1.37

[1/] Different lowercase letters indicate statistically significant differences among the studied lines within the same column ($p < 0.05$).

Table 5. Growth performance of selected Shan tea lines under nursery conditions at 6 months after propagation in the winter-spring season

Parameters / Genotype	Shoot length (cm)	Seedling stem diameter (mm)	Fresh root weight (g)	Shoot biomass (g)	Leaf weight (g)	Number of primary roots
LC.TT5	5.94±0.96$^{a/1}$	1.70±0.12^{b}	0.24±0.11^{b}	0.62±0.08^{ab}	0.32±0.05^{ab}	3.40±0.89^{a}
LC.TT11	5.60±1.40^{a}	1.64±0.05^{b}	0.17±0.07^{bc}	0.56±0.11^{bc}	0.31±0.07^{ab}	3.60±0.89^{a}
LC.TT16	6.48±1.03^{a}	1.80±0.10^{b}	0.20±0.06^{bc}	0.72±0.11^{a}	0.36±0.04^{a}	3.80±1.10^{a}
YB.SB4	6.24±1.57^{a}	2.04±0.11^{a}	0.38±0.08^{a}	0.59±0.03^{bc}	0.31±0.03^{ab}	4.20±1.30^{a}
HG.LT1	4.98±1.16^{a}	1.78±0.08^{b}	0.11±0.03^{c}	0.47±0.06^{c}	0.27±0.02^{b}	2.80±0.84^{a}
CV %	20.38	6.58	27.77	14.91	14.31	29.33
$LSD_{0,05}$	1,60	0.16	0.08	0.12	0.06	1.40

[1/] Different lowercase letters indicate statistically significant differences among the studied lines within the same column ($p < 0.05$).

Table 6. Growth performance of selected Shan tea lines under nursery conditions at 10 months after propagation in the winter-spring season

Parameters / Genotype	Shoot length (cm)	Seedling stem diameter (mm)	Fresh root weight (g)	Shoot biomass (g)	Leaf weight (g)	Number of primary roots
LC.TT5	36.20±4.51$^{b/1}$	3.58±0.26^{ab}	1.38±0.54^{b}	5.05±0.78^{bc}	2.53±0.38^{b}	6.83±1.64^{a}
LC.TT11	29.10±2.35^{c}	3.38±0.37^{b}	1.37±0.15^{b}	4.35±0.58^{c}	2.66±0.65^{b}	6.27±0.84^{a}
LC.TT16	39.64±1.83^{a}	4.04±0.17^{a}	1.78±0.17^{b}	5.82±0.61^{ab}	3.80±0.41^{a}	6.62±1.52^{a}
YB.SB4	39.82±2.60^{a}	3.95±0.11^{a}	1.41±0.11^{b}	5.90±0.55^{ab}	3.28±0.66^{a}	6.36±0.71^{a}
HG.LT1	35.12±2.29^{b}	3.76±0.13^{ab}	1.43±0.42^{b}	6.38±0.56^{a}	3.45±0.40^{a}	6.50±0.89^{a}
CV %	5.28	8.37	15.66	11.43	13.83	17.11
$LSD_{0,05}$	2.55	0.42	0.31	0.84	0.58	1.48

[1/] Different lowercase letters indicate statistically significant differences among the studied lines within the same column ($p < 0.05$).

Figure 2. Selected shan tea lines at 6 months after propagation in the summer-autumn season: (A) LC.TT5, (B) LC.TT11, (C) LC.TT16, (D) YB.SB4, (E) HG.LT1

Figure 3. Selected shan tea lines at 6 months after propagation in the winter-spring season: (A) LC.TT5, (B) LC.TT11, (C) LC.TT16, (D) YB.SB4, (E) HG.LT1

The results showed differences in growth and developmental potential among the selected Shan tea lines propagated by cuttings in the summer-autumn and winter-spring seasons (Tables 3-6). Growth indicators such as shoot length, seedling stem diameter, and biomass weight (whole plants, leaves, roots) gradually increased with the age of the plant, reflecting the traditional growth pattern of tea plants in nursery conditions Notably, early root system development plays a critical role, directly influencing the survival and subsequent growth of the cuttings.

In the summer-autumn season, clearly differences among the lines were observed at both evaluation stages. At 6 months old, shoot length ranged from 4.74 to 6.08 cm, while fresh root weight ranged from 0.14 to 0.34 g (Table 3 and Figure 2). By 10 months old, these indicators increased markedly, with shoot length reaching 28.80–37.44 cm and leaf shoot weight from 4.53 to 5.96 g (Table 4 and Figure 4). Among the studied lines, the LC.TT16 line demonstrated outstanding and stable advantages in growth and biomass accumulation during the summer-autumn crop.

A similar trend was observed in the winter-spring season, although overall growth performance was slightly improved. At 6 months old, the shoot length ranged from 4.98 to 6.48 cm, while the fresh root weight ranged from 0.11 to 0.38 g (Table 5 and Figure 3). By 10 months old, the indicators increased significantly, with shoot length from 29.10 to 39.82 cm and leaf shoot weight from 4.35 to 6.38 g (Table 6 and Figure 5). Notably, LC.TT16 and YB.SB4 consistently maintained higher and more stable values, indicating superior growth potential under winter-spring conditions.

Figure 4. Selected Shan tea lines at 10 months after propagation in the summer-autumn season: (A) LC.TT5, (B) LC.TT16, (C) YB.SB4

Figure 5. Selected Shan tea lines at 10 months after propagation in the winter-spring season: (A) LC.TT5, (B) LC.TT11, (C) LC.TT16, (D) YB.SB4, (E) HG.LT1

Effect of substrate on survival, rooting, and growth of selected shan tea cuttings

The effects of three substrate treatments, CT1: pure red-yellow ferralitic soil; CT2: 10% sand + red-yellow ferralitic soil; and CT3: 5% sand + 10% rice husk ash + red-yellow ferralitic soil on cutting propagation performance of the selected Shan tea line LC.TT16 were evaluated (Table 7).

Table 7. Effect of substrate on survival, rooting ability, and nursery performance of cuttings from shan tea line LC.TT16

CT	After 2 months				After 10 months	
	Survival rate (%)	**Callus formation rate (%)**	**Rooting rate (%)**	**Bud emergence rate (%)**	**Survival rate (%)**	**Transplantable seedling rate (%)**
CT1	92.00±2.00$^{b/1}$	85.33±1.16^{b}	80.00±2.00^{b}	81.33±1.16^{b}	75.33±1.16^{b}	66.00±2.00^{b}
CT2	93.33±1.16^{ab}	88.67±3.06^{ab}	83.33±3.06^{ab}	84.00±2.00^{ab}	78.00±2.00^{ab}	68.67±2.31^{ab}
CT3	95.33±1.16^{a}	90.00±2.00^{a}	85.33±1.16^{a}	86.00±2.00^{a}	80.00±2.00^{a}	72.00±2.00^{a}
CV%	0.71	1.86	1.27	2.11	0.86	2.26
LSD	0.015	0.037	0.024	0.040	0.015	0.035

[1/] Different lowercase letters indicate statistically significant differences among treatments within the same column ($p < 0.05$).

The results showed that growth parameters of tea cuttings at 2 months after propagation in the three different substrate treatments, indicated that the survival rate after rooting ranged from 92.00% to 95.33%, the highest in CT3 (95.33%) and lowest in CT1 (92.00%) as seen in Table 7. A similar trend was observed across subsequent parameters, including callus formation rate 85.33%–90.00%, root emergence rate 80.00%–85.33%, bud emergence rate 81.33%–86.00%, survival rate after 10 months 75.33%–80.00%, and transplantable seedling rate 66.00%–72.00%. Notably, the addition of sand and particularly rice husk ash increased the transplantable seedling rate by approximately 6% compared to the use of pure red-yellow ferralitic soil. The growth indicators across different stages are shown in Tables 8-9 and Figures 6 -7.

Figure 6. Root development of the selected shan tea line at 6 months after planting under different substrates: (A) CT1, (B) CT2, (C) CT3

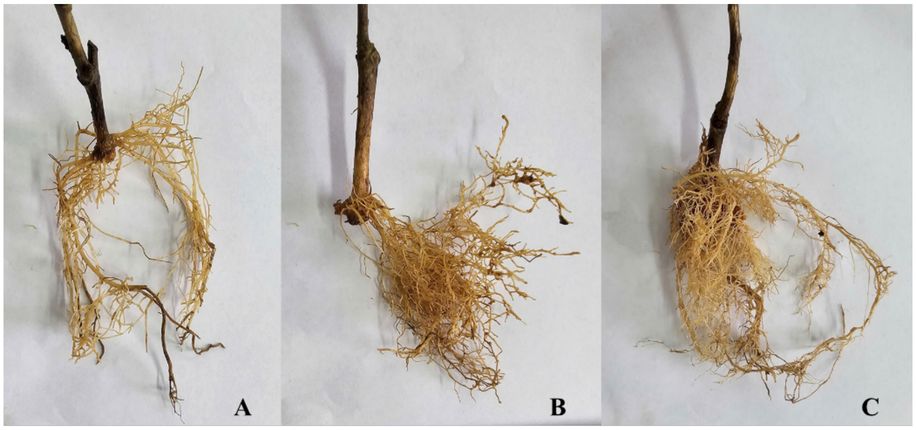

Figure 7. Root development of the selected shan tea line at 10 months after planting under different substrates: (A) CT1, (B) CT2, (C) CT3

Table 8. Growth and biomass parameters of shan tea line LC.TT16 at 6 months after propagation

CT	Shoot length (cm)	Shoot, leaf biomass (g)	Seedling stem diameter (mm)	Fresh root weight (g)	Dry root weight (g)	Number of primary roots
CT1	6.48±1.03[a/1]	0.69±0.09[a]	1.78±0.13[a]	0.20±0.06[b]	0.08±0,01[a]	3.80±1.09[a]
CT2	6.64±0.81[a]	0.72±0.10[a]	1.86±0.21[a]	0.24±0.04[ab]	0.08±0.01[a]	3.80±0.82[a]
CT3	6.78±0.97[a]	0.76±0.06[a]	1.92±0.13[a]	0.28±0.04[a]	0.10±0.01[a]	4.05±1.10[a]
CV %	16.86	11.89	4.29	20.37	11.74	28.72
$LSD_{0,05}$	1.63	0.13	0.12	7.11	1.51	2.36

[1/] Different lowercase letters indicate statistically significant differences among treatments within the same column ($p < 0.05$).

Table 9. Morphological growth and biomass parameters of shan tea line LC.TT16 at 10 months after propagation

	Shoot length (cm)	Shoot, leaf biomass (g)	Seedling stem diameter (mm)	Fresh root weight (g)	Dry root weight (g)	Number of primary roots
CT1	39.44±2.14[a/1]	5.68±0.47[b]	3.84±0.30[a]	1.52±0.18[b]	0.35±0.04[b]	6.17±1.17[a]
CT2	40.42±1.82[a]	6.06±0.52[ab]	3.90±0.26[a]	1.80±0.25[ab]	0.39±0.05[b]	6.33±1.03[a]
CT3	42.32±2.58[a]	6.49±0.48[a]	4.00±0.35[a]	1.97±0.35[a]	0.56±0.09[a]	6.83±0.98[a]
CV %	6.09	7.72	8.61	17.90	16.32	10.18
$LSD_{0,05}$	3.62	0.68	0.49	0.46	0.10	0.96

[1/] Different lowercase letters indicate statistically significant differences among treatments within the same column ($p < 0.05$).

Discussion

Effect of propagation season on survival, rooting, and nursery performance of Shan tea cuttings

Previous studies on *Camellia sinensis* indicated that adventitious root formation is regulated by the expression of genes associated with auxin signaling and cell division. Differences among genotypes in tissue regeneration capacity and rooting ability lead to variation in propagation efficiency (Liu *et al*., 2023; Zhou *et al*., 2025a). The YB.SB4 and LC.TT16 lines also exhibited higher bud emergence rates (77.33–78.00%) and correspondingly higher survival rates (72.67–74.00%), whereas LC.TT5 showed lower performance in both traits. This suggests that the coordination between root formation and shoot development is a key determinant of seedling survival.

Recent studies on the molecular biology of root formation in *Camellia sinensis* show that this process is controlled by the expression of genes related to auxin signaling and cell division, especially the ARF, GH3, and LBD gene groups; the different expression levels among varieties lead to significant differences in rooting rates (Liu *et al*., 2023; Wang *et al*., 2024). These findings are consistent with the relationship observed in the present study between callus formation and rooting rate, suggesting that the intrinsic regenerative capacity of each genotype plays a more decisive role than technical factors in determining propagation success. Regarding the relationship between rooting and sprouting, LC.TT16 exhibited the highest sprouting rate (83.33%) and the highest survival rate after 10 months (76.00%). This indicates that the formation of roots and shoots is correlated. Conversely, the LC.TT5 line has a lower sprouting rate (76.67%) and the lowest survival rate (70.67%). The rooting rate exceeds 75%, but the transplantable seedling rate is only 61–67%. Previous studies have shown that the survival rate after planting in soil is always lower than the rooting rate in tea seedlings (Zhang *et al*., 2025). This is related to the high transpiration demand and water stress during the development of young shoots (Shrestha and Miles, 2025). Young tea plants have an underdeveloped cutin layer and unstable stomatal regulation, making them very sensitive to radiation and water deficiency. Therefore, the success of cuttings depends not only on root formation but also on physiological adaptation after rooting (Zhou *et al.,* 2025a). Additionally, semi-hardwood cuttings taken from the middle portion of shoots tend to exhibit better rooting and growth performance (Shrestha and Miles, 2025).

Effect of propagation season on the growth of Shan tea cuttings in the nursery stage

The results suggested that the winter-spring season, characterized by cooler temperatures, more stable humidity, and lower radiation, provides optimal conditions for adventitious root formation and vegetative growth in Shan tea. Recent physiological studies also emphasize that a favorable environment provides optimal conditions for and promotes cell division at the base of the cuttings, thereby enhancing the ability to form adventitious roots and overall cutting efficiency (Liu *et al*., 2023). This process is regulated by endogenous plant hormones, particularly auxin, which plays a central role in controlling cell division and differentiation during adventitious root development (Wang *et al.,* 2024).

The significant differences between the lines are consistently observed throughout both cropping seasons. The LC.TT16 and YB.SB4 lines continuously achieve the highest growth indicators in both seasons, including those related to the root system such as root weight and the number of first–order roots. This indicates that these two lines have strong growth potential and good cutting rooting ability, suitable for a biomass distribution model that prioritizes the root system in the initial stage, creating a foundation for more efficient nutrient and water absorption when transplanted. In contrast, HG.LT1 and LC.TT11 exhibited relatively lower growth performance, possibly due to limitations in adventitious root formation or less efficient biomass allocation (Wang *et al*., 2024).

Physiologically, the results showed that a well–developed root system accompanied by balanced biomass distribution between roots and shoots is a key factor that helps young tea plants increase their ability to absorb nutrients and water, maintaining stable growth after transplanting. A strong root system also enhances resistance to environmental stress after planting in the field, which is an important factor in producing high–quality seedlings. Recent studies in other plant species have similarly demonstrated that the coordination of endogenous hormones-particularly auxin-together with enzymatic processes involved in energy metabolism plays a key role in rooting efficiency and overall cutting performance (Zhou *et al.,* 2025b).

Overall, the results indicated that LC.TT16 and YB. SB4 exhibited the greatest growth potential due to its strong capacity for adventitious root formation and efficient biomass allocation. The winter-spring season in northern Vietnam is an advantageous season for cutting shan tea. This is provided an important scientific basis for prioritizing the selection of these lines for the vegetative propagation program and the production of elite shan tea varieties, as well as opening up further research directions on hormone mechanisms and gene regulation of tea cuttings under Vietnamese climatic conditions.

Effect of substrate on survival, rooting, and growth of selected shan tea cuttings

These differences can largely be attributed to the physical characteristics of the substrates. Pure red-yellow ferralitic soil (CT1) is typically compact, with poor drainage and limited aeration, which can hinder root formation. When sand was added (CT2), the substrate became more porous, leading to a modest increase in rooting rate from 80.00% to 83.33%. The most pronounced effect was observed in CT3, where roasted rice husk ash creates an environment that is both well-aerated and moisture-retentive, aiding callus and root development.

These advantages continued into later stages of growth. CT3 consistently produced higher bud emergence (86.00%) and a greater survival rate after 10 months (80.00%) compared to CT1 (81.33% and 75.33%, respectively). This suggests that root systems formed in a looser, more porous substrate are better able to function efficiently and maintain water balance during shoot development. As a result, the transplantable seedling rate in CT3 reached 72.00%, surpassing that of CT2 (68.67%) and CT1 (66.00%). Taken together, these findings are highlighted the importance of improving substrate aeration and moisture retention. The addition of sand and rice husk ash not only enhanced rooting performance but also improved overall seedling quality. Under nursery conditions, CT3 therefore are appeared to be the most suitable substrate for vegetative propagation of shan tea. Previous studies have also demonstrated that tea roots are highly sensitive to oxygen deficiency; substrates with higher porosity enhance root respiration and auxin transport, thereby improving rooting capacity and seedling growth (Teshome *et al*., 2016).

The results showed that the substrate component significantly affected the growth and rooting ability of the selected Shan tea line LC.TT16, as indicated by parameters such as shoot length, shoot-root biomass, stem diameter, and the number of primary roots. This effect is consistently reflected across key parameters, including shoot length, shoot and root biomass, stem diameter, and the number of primary roots. Overall, these findings are emphasized the importance of substrate physical properties-particularly porosity, aeration, and water-holding capacity-in regulating adventitious root formation and biomass allocation during the nursery stage.

During the 6-month period, the growth indicators of the shoot (shoot length, plant weight, stem diameter) among the treatments CT1–CT3 was not significantly differed ($p > 0.05$), indicating that in the early stage, shoot growth of the tea cuttings mainly is depended on the internal reserves of the cuttings rather than the substrate conditions. This result aligns with the understanding that during the root initiation stage, carbohydrates and endogenous hormones within the cuttings play a key role in initial shoot growth (Chen *et al*., 2024).

However, over the 10-month period, CT3 (5% sand + 10% rice husk ash + yellow-red ferralitic soil) had the longest shoot length, the highest plant weight, and the greatest stem diameter, with statistically significant differences compared to CT1 (pure yellow-red ferralitic soil) ($p < 0.05$). This indicates that once the root system is fully developed, a growing medium rich in porosity and aeration will promote water and nutrient absorption, thereby enhancing aboveground growth. The differences among treatments are most evident in root-related parameters. At both 6 and 10 months, CT3 showed significantly higher fresh root weight, dry root weight, and the number of primary roots compared to CT1, with many indicators reaching statistical significance ($p < 0.05$). These results demonstrate that the addition of sand and rice husk ash substantially improves the root environment, particularly by increasing oxygen availability and drainage capacity.

CT3 is not only increased the absolute biomass of the roots but also improved the distribution of biomass between roots and shoots, as evidenced by a higher root/shoot ratio compared to CT1. This balanced biomass distribution is an important factor in determining the quality of tea seedlings which is helped the young plants adapt better when transplanted into the field. The increase in both the number and biomass of roots in CT3 demonstrated that biochar not only improved the physical conditions of the substrate but also promoted the biological activity of the root system. These results are entirely consistent with the understanding of the role of biological materials in improving soil structure and stimulating the growth of plant root systems (Dalimoenthe, 2013; Lehmann *et al*., 2015; McConnaughey, 2013; Shrestha and Miles, 2025; Teshome *et al*., 2016). Improving root quality during the nursery stage is particularly significant for growth in the field, as a well-developed root system helps seedlings absorb water and nutrients more effectively, withstand drought better, and recover quickly after planting.

Sand improves aeration and reduces soil compaction, thereby limiting waterlogging, while rice husk ash is provided a porous structure with high moisture retention, supplies silicon, and may help to suppress soil-borne pathogens. This combination is created a balanced environment for the substrate in terms of water, air, and nutrients, facilitating the process of adventitious root formation. Similar findings have been reported in other studies, where substrates containing porous organic materials such as rice husk or biochar improved rooting percentage and primary root development, confirming that substrate aeration is a key determinant of successful cutting propagation in tea (Kartika *et* *al*., 2021; Shrestha and Miles, 2025) and other plant species (Adzraku *et al.,* 2017; Walianggen, 2022).

It is concluded that both propagation season and substrate composition are found to be critical factors influencing the efficiency of vegetative propagation and seedling growth of selected shan tea (*Camellia sinensis* var. *assamica*) under nursery conditions in northern Vietnam. The results showed that propagation season and genotype significantly influenced cutting performance, and moderate temperature and stable humidity create a favorable environment for adventitious root formation and seedling development. The use of a mixed substrate consisting of red-yellow ferralitic soil, sand, and rice husk ash are significantly improved growth performance as compared to pure ferralitic soil. This was evident through increased in shoot length, root biomass, number of primary roots, and stem diameter. These results are highlighted the role of aeration, water balance, and porosity of the substrate in stimulating and supporting root system growth.

In addition, the significant differences observed among tea lines indicated that genetic factors have a clear influence on rooting ability and biomass distribution, with some lines demonstrating high stability across different seasons and substrate conditions. Based on the obtained results, the study is recommended the prioritizing cuttings during the winter-spring season and using a substrate of red-yellow ferralitic soil + 5% sand + 10% rice husk ash to optimize the production of high-quality Shan tea seedlings. These results are provided a valuable scientific basis for improving vegetative propagation practices and contributed to the sustainable development and conservation of ancient Shan tea genetic resources in Northern Vietnam.

Acknowledgments

This research is funded by Hanoi Pedagogical University 2 under grant number HPU2.2023-UT-16 to Hong Viet La.

Conflicts of interest

The authors declare no conflict of interest.

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(Received: 13 April 2026, Revised: 4 July 2026, Accepted: 6 July 2026)