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Effects of Standardized Cutting Propagation on Survival and Prickle-Free Trait Retention in Green Sichuan Pepper

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Abstract

To address the low propagation efficiency of superior prickle-free green Sichuan pepper plants and prickle reappearance in their seed-derived offspring, separate single-factor experiments on hardwood and softwood cuttings were conducted in 2025-2026 using selected superior plants. The experiments examined the effects of cutting specifications or degree of lignification, rooting treatments, and substrate mixtures on cutting survival, root number, root length, and root diameter. Field assessment one year after transplanting was used to evaluate prickle-free trait retention in vegetatively propagated plants. The single-factor experiments showed that, for hardwood cuttings, one-year-old dormant shoots 15 - 20 cm long with 3 - 4 plump buds, soaking in 100 $mg \cdot L^{-1}$ ABT for 3 h, and a humus-rich soil:river sand:composted organic fertilizer substrate (2:2:1) performed well in their respective experiments. The 100 $mg \cdot L^{-1}$ ABT treatment for 3 h achieved 85.0% survival, with a mean of 12.3 roots, a root length of 8.76 cm, and a root diameter of 0.348 cm. For softwood cuttings, semi-lignified current-season shoots, soaking in 30 $mg \cdot L^{-1}$ ABT for 1.5 h, and a river sand:perlite:vermiculite substrate (1:1:1) performed well in their respective experiments. The 30 $mg \cdot L^{-1}$ ABT treatment for 1.5 h achieved 82.0% survival, with a mean of 15.2 roots and a root length of 6.16 cm. One year after transplanting, prickle-free trait retention was 100% in both hardwood- and softwood-cutting-derived plants, compared with only 65.0% in seed-derived plants transplanted at the same time, which had a prickle reappearance rate of 35.0%. These observations provide preliminary evidence that the standardized cutting propagation system established in this study can significantly improve nursery survival while maintaining the prickle-free trait, providing a technical basis for large-scale propagation of this superior clone.

Keywords

Prickle-Free Green Sichuan Pepper, Cutting Propagation, ABT Rooting Powder, Substrate Mixture, Trait Stability

1. Introduction

Sichuan pepper comprises deciduous shrubs or small trees in the family Rutaceae. Its fruit pericarp is used as a traditional spice and medicinal material in China and has considerable economic value and ecological benefits [1]. Green Sichuan pepper is mainly *Z. armatum* DC., which is widely cultivated in Sichuan and Chongqing and is an important specialty tree crop for rural revitalization [2]. Traditionally cultivated prickly varieties have densely prickled branches, making harvesting difficult and increasing occupational injuries. Harvest labor accounts for more than 50% of total costs and has become a constraint on large-scale development of the Sichuan pepper industry [3].

Prickle-free or sparsely prickled Sichuan pepper originates from mutant individuals found in natural populations. These plants have reduced prickles, a high proportion of fruiting branches, and lower harvesting labor requirements. In recent years, prickle-free or sparsely prickled clones, including Guang'an Green Sichuan Pepper No. 1, Kuntai No. 2, the Wuxuan series, and the Jijiao series, have been selected in Sichuan, Chongqing, Gansu, Yunnan, and Hebei and have performed well in production [4]. Through integrated multi-omics analysis, He *et al.* (2026) elucidated the molecular regulatory network of prickle development in *Z. armatum* and identified the regulatory roles of key genes, including *JAR4* and *MYC2*, providing a theoretical basis for molecular breeding of prickle-free Sichuan pepper [5]. However, the prickle-free trait is a favorable clonal variation, and genetic recombination and trait segregation during seed propagation cause substantial prickle reappearance in the offspring. Grafting can retain maternal traits, but limited rootstock availability and the labor required restrict propagation efficiency and make it difficult to meet the demand for high-quality planting material [6].

Cutting propagation is a major method for rapid clonal multiplication of superior woody plants. It offers a high multiplication rate, uniform planting material, and genetic consistency between offspring and mother plants [7]. Sichuan pepper is a callus-rooting species, and cutting rooting is affected by mother-plant age, shoot lignification, auxin treatment, and the physical and chemical properties of the substrate [8]. Previous studies reported relatively high survival in hardwood cuttings approximately 20 cm long after soaking in 100 mg·L^{-1} ABT for 2 h. For softwood cuttings, semi-lignified shoots treated with suitable concentrations of mixed NAA/IBA achieved rooting percentages above 70% [9]. Wei *et al.* found that exogenous hormone treatments significantly increased rooting in Sichuan pepper cuttings and promoted adventitious root formation by increasing nutrient

contents and related enzyme activities within the cuttings [9]. Most studies, however, have used prickly Sichuan pepper or common cultivated varieties. Systematic research on cutting protocols for the superior prickle-free green Sichuan pepper clone remains limited, particularly field evidence of prickle-free trait retention over multiple years.

Using selected superior prickle-free green Sichuan pepper plants, this study compared cutting specifications or degree of lignification, rooting agent types and concentrations, and substrate mixtures in separate single-factor randomized block experiments for hardwood and softwood cuttings. The aim was to identify standardized nursery propagation procedures and evaluate prickle-free trait retention through field assessment one year after transplanting.

2. Materials and Methods

2.1. Study Site

The study was conducted in 2025-2026 at the Green Sichuan Pepper Modern Agricultural Park in Qianfeng District, Guang'an, Sichuan Province, China. The site is in the hilly eastern Sichuan Basin (106˚38'E, 30˚22'N), at approximately 320 m above sea level. It has a humid subtropical monsoon climate, with a mean annual temperature of 17.8˚C, annual precipitation of approximately 1050 mm, and a frost-free period of approximately 300 days. The nursery is level, with convenient irrigation and drainage. Its purple soil has a pH of 6.8 - 7.2 and is suitable for growing green Sichuan pepper planting material [10].

2.2. Plant Materials

Mother plants were selected from vigorous, pest- and disease-free superior prickle-free green Sichuan pepper plants in the park after three consecutive years of field observation. They were 3 - 5 years old, with completely regressed prickles and a fruiting-branch proportion ≥80%. The plants were spaced at 3 m × 4 m and received routine water and fertilizer management. Hardwood cuttings were collected in December 2024, during dormancy after leaf fall, from fully lignified one-year-old branches in the middle and upper crown. Softwood cuttings were collected from current-season shoots on the crown periphery in June 2025, during vigorous growth [11].

The rooting agent was ABT rooting powder (Beijing ABT Biotechnology Co., Ltd.). Substrate materials included humus-rich soil (local forest topsoil with organic matter ≥20%), river sand (passed through a 2 mm sieve, washed, and air-dried), perlite (3 - 5 mm particle size), vermiculite (2 - 4 mm particle size), and composted organic fertilizer (cattle manure:straw = 3:1, composted for 12 months, with organic matter ≥30%).

2.3. Experimental Design

Hardwood and softwood cutting experiments were conducted separately using single-factor randomized block designs. Three independent parallel experiments

were conducted for each cutting type. Only the factor under investigation was varied in each experiment, while the other conditions were kept constant. Each replicate contained 100 cuttings.

Three factors were examined in hardwood cuttings. (1) Cutting specifications (A): A1, 10 - 15 cm long with 2 - 3 buds; A2, 15 - 20 cm long with 3 - 4 plump buds; and A3, 20 - 25 cm long with 4 - 5 buds. (2) Rooting treatments (B): B1, water control; B2, 50 mg·L^{-1} ABT soaking for 2 h; B3, 100 mg·L^{-1} ABT soaking for 3 h; and B4, 500 mg·L^{-1} ABT quick dip for 10 s. (3) Substrate mixtures (C): C1, humus-rich soil:river sand = 1:1; C2, humus-rich soil:river sand:composted organic fertilizer = 2:2:1; and C3, river sand:perlite = 1:1.

Three factors were examined in softwood cuttings. (1) Degree of shoot lignification (D): D1, non-lignified tender shoots; D2, semi-lignified current-season shoots; and D3, lignified current-season shoots. (2) Rooting treatments (E): E1, water control; E2, 20 mg·L^{-1} ABT soaking for 1 h; E3, 30 mg·L^{-1} ABT soaking for 1.5 h; and E4, 100 mg·L^{-1} ABT quick dip for 5 s. (3) Substrate mixtures (F): F1, river sand only; F2, river sand:perlite:vermiculite = 1:1:1; and F3, humus-rich soil:river sand = 1:1.

2.4. Cutting Preparation and Nursery Management

Hardwood cuttings were planted in mid-February 2025, when spring temperatures were rising and soil temperature remained above 10˚C. The upper cut was made horizontally approximately 1 cm above the terminal bud, and the lower cut was made at a 45˚ angle. After the prescribed soaking treatments, cuttings were inserted into nursery beds at 10 cm × 15 cm spacing to one-third of their length. The beds were thoroughly watered and covered with plastic mulch. Softwood cuttings were planted on an overcast day in late June 2025. Cuttings were 10 - 15 cm long, with 2 - 3 upper leaves retained and each leaf trimmed by half. They were inserted at 5 cm × 10 cm spacing into separate nursery beds containing river sand, river sand + perlite + vermiculite, or humus-rich soil + river sand, using the substrate ratios described above. Insertion depth was approximately half the cutting length. The beds were thoroughly watered and covered with a shade structure providing 50% - 60% shade and a low plastic tunnel [12].

Routine nursery management was applied. For hardwood cuttings, soil water content was maintained at 60% - 70% of field capacity. For softwood cuttings, air relative humidity in the low plastic tunnel was maintained at 85% - 90%, then gradually reduced to 70% - 80% through ventilation and hardening after rooting. Temperature under cover was maintained at 20˚C - 25˚C; shading and misting were used when it exceeded 30˚C. After rooting, a foliar mixture of 0.2% urea and 0.2% potassium dihydrogen phosphate was applied every 15 days for 2 - 3 applications. Weeds were removed promptly, and damping-off, root rot, aphids, and spider mites were controlled [13].

2.5. Measurements and Assessment

Survival was assessed 60 days after planting. Survival (%) = number of surviving

cuttings/number of planted cuttings × 100%. Thirty surviving cuttings were randomly selected from each replicate. Their roots were washed, and root number, mean root length, and root diameter 1 cm from the root base were measured using a ruler and a vernier caliper. In early October 2025, surviving plants from the treatments were transplanted to the field at the same density when they reached 30 - 40 cm in height and a basal stem diameter ≥0.5 cm. Seed-derived plants raised from seeds of the same superior mother plants were transplanted as controls. One year after transplanting, in October 2026, each plant was assessed for prickles in the field. Stem sections ≥5 cm long on all current-season shoots were examined. A plant was classified as prickle-free if its total prickle count was ≤3 and it had no hard prickles ≥2 mm long. The same criterion was applied to cutting-derived and seed-derived plants. Prickle-free trait retention (%) = number of plants meeting the above criterion/total number of plants assessed × 100% [14].

2.6. Data Analysis

Data were organized in Excel 2019 and analyzed by one-way analysis of variance (ANOVA) in SPSS 26.0. Duncan's multiple range test was used for multiple comparisons at $P < 0.05$. Data in the tables are presented as mean ± standard deviation.

3. Results and Analysis

3.1. Hardwood Cuttings

Cutting specifications, rooting treatments, and substrate mixtures all significantly affected survival and rooting of prickle-free green Sichuan pepper hardwood cuttings. The effect of rooting treatment was highly significant ($P < 0.01$), while cutting specifications and substrate mixtures had significant effects ($P < 0.05$).

Table 1. Effects of cutting specifications on survival and rooting of prickle-free green Sichuan pepper hardwood cuttings.

Treatment	Cutting specifications	Survival (%)	Root number	Mean root length (cm)	Basal root diameter (cm)
A1	10 - 15 cm, 2 - 3 buds	63.3c	8.5c	5.90c	0.243c
A2	15 - 20 cm, 3 - 4 buds	74.7a	11.3a	7.78a	0.315a
A3	20 - 25 cm, 4 - 5 buds	68.0b	9.7b	6.44b	0.278b

Note: Different lowercase letters within a column indicate significant differences among treatments ($P < 0.05$). The same notation applies to the following tables.

A2 had the highest survival, at 74.7%, which was 11.4 and 6.7 percentage points higher than A1 and A3, respectively. Based on the single-factor results, A2 was provisionally recommended in terms of root number, root length, and root diameter. A1 cuttings were short and had limited nutrient reserves, making it difficult to support both rooting and bud growth. A3 cuttings were excessively long, increasing the transpiring surface and the risk of water loss during dormancy. This agrees with Yang *et al.*, who found that suitable cutting specifications provided an

optimal balance between nutrient supply and transpirational water loss in miniature hardwood cuttings of Sichuan pepper [7] (Table 1).

Table 2. Effects of rooting treatments on survival and rooting of prickle-free green Sichuan pepper hardwood cuttings.

Treatment	Rooting treatment	Survival (%)	Root number	Mean root length (cm)	Basal root diameter (cm)
B1	Water (CK)	44.3d	5.1d	4.11d	0.182d
B2	50 mg·L^{-1} ABT, soaking for 2 h	73.3c	9.6c	6.99c	0.291c
B3	100 mg·L^{-1} ABT, soaking for 3 h	85.0a	12.3a	8.76a	0.348a
B4	500 mg·L^{-1} ABT, quick dip for 10 s	80.0b	11.2b	8.12b	0.323b

Survival in the water control B1 was only 44.3%, indicating weak inherent rooting capacity in hardwood cuttings. ABT treatments significantly improved all measured indicators. B3 (100 mg·L^{-1} soaking for 3 h) had the highest survival, reaching 85.0% in the single-factor rooting-treatment experiment, with 12.3 roots and a root length of 8.76 cm. These results supported the recommended treatment combination from the single-factor experiment. The B4 quick-dip treatment was slightly less effective than B3, possibly because high-concentration, short-duration treatment caused uneven adsorption of the agent at the cut surface. Yang *et al.* (2021) also confirmed that suitable concentrations of exogenous auxin significantly increased rooting in Sichuan pepper hardwood cuttings by promoting cambial cell division and root primordium differentiation [7] (Table 2).

C2 had the highest survival, at 79.7%, and was provisionally recommended based on its rooting indicators in the single-factor experiment. C3 (river sand:perlite) had good aeration but poor water and nutrient retention, making cuttings prone to water loss. C1 had insufficient nutrient retention. These findings are similar to the substrate results of Yang *et al.* (2021) for Sichuan pepper hardwood cuttings: a well-proportioned mixed substrate better balances air and water and provides a stable microenvironment for rooting [7] (Table 3).

Table 3. Effects of substrate mixtures on survival and rooting of prickle-free green Sichuan pepper hardwood cuttings.

Treatment	Substrate mixture	Survival (%)	Root number	Mean root length (cm)	Basal root diameter (cm)
C1	Humus-rich soil:river sand = 1:1	68.3b	8.9b	6.32b	0.272b
C2	Humus-rich soil:river sand:composted organic fertilizer = 2:2:1	79.7a	12.0a	8.14a	0.333a
C3	River sand:perlite = 1:1	61.3c	7.6c	5.20c	0.221c

3.2. Softwood Cuttings

D2 semi-lignified current-season shoots had the highest survival, at 81.0%, and 15.3 roots, significantly exceeding D1 and D3. Semi-lignified shoots had active cambial cell division and high rooting potential, while possessing sufficient mechanical strength. Their resistance to microbial infection and environmental stress was greater than that of non-lignified tender shoots. In D3, a higher degree of lignification reduced cell division capacity and slowed rooting (Table 4).

Table 4. Effects of shoot lignification on survival and rooting of prickle-free green Sichuan pepper softwood cuttings.

Treatment	Shoot type	Survival (%)	Root number	Mean root length (cm)	Basal root diameter (cm)
D1	Non-lignified tender shoots	52.0c	11.6c	4.31c	0.177c
D2	Semi-lignified current-season shoots	81.0a	15.3a	6.28a	0.259a
D3	Lignified current-season shoots	65.3b	12.5b	5.14b	0.218b

In the single-factor rooting-treatment experiment, survival in the water control E1 was only 39.0%. E3 (30 mg·L^{-1} ABT soaking for 1.5 h) achieved the highest survival, at 82.0%, with 15.2 roots. The E4 quick-dip treatment was less effective than E3, indicating that low-concentration, long-duration soaking was preferable for softwood cuttings (Table 5).

Table 5. Effects of rooting treatments on survival and rooting of prickle-free green Sichuan pepper softwood cuttings.

Treatment	Rooting treatment	Survival (%)	Root number	Mean root length (cm)	Basal root diameter (cm)
E1	Water (CK)	39.0d	6.5d	3.53d	0.148d
E2	20 mg·L^{-1} ABT, soaking for 1 h	70.0c	13.8c	5.38c	0.231c
E3	30 mg·L^{-1} ABT, soaking for 1.5 h	82.0a	15.2a	6.16a	0.261a
E4	100 mg·L^{-1} ABT, quick dip for 5 s	64.7b	12.1b	4.80b	0.214b

Table 6. Effects of substrate mixtures on survival and rooting of prickle-free green Sichuan pepper softwood cuttings.

Treatment	Substrate mixture	Survival (%)	Root number	Mean root length (cm)	Basal root diameter (cm)
F1	River sand only	58.3c	10.6c	4.67c	0.191c
F2	River sand:perlite: vermiculite = 1:1:1	77.7a	14.7a	6.04a	0.249a
F3	Humus-rich soil:river sand = 1:1	65.3b	12.1b	5.21b	0.218b

Softwood cuttings require good substrate aeration. F2 (river sand:perlite:vermiculite = 1:1:1) had the highest survival, at 77.7%. F3 (humus-rich soil:river sand) had poorer aeration, making the cut surfaces of softwood cuttings prone to rot under hot and humid conditions. F1, river sand alone, had poor water retention, making leaves prone to water loss and wilting (Table 6).

3.3. Prickle-Free Trait Stability after Transplanting

Field assessment of individual plants one year after transplanting showed that both hardwood- and softwood-cutting-derived plants met the prickle-free classification criterion, with 100% prickle-free trait retention. Approximately 35% of seed-derived plants transplanted at the same time developed conspicuous prickles on one-year-old branches, resulting in prickle-free trait retention of only approximately 65% (Table 7).

Table 7 Prickle-free trait retention in green Sichuan pepper one year after transplanting under different propagation methods.

Propagation method	Plants assessed	Prickle-free plants	Prickle-free trait retention (%)	Prickle reappearance (%)
Hardwood cuttings	200	200	100.0a	0.0a
Softwood cuttings	200	200	100.0a	0.0a
Seed propagation	200	130	65.0b	35.0b

4. Discussion

Cutting specifications and the degree of lignification determine internal nutrient reserves and cellular regeneration capacity. In this study, hardwood cuttings 15 - 20 cm long and semi-lignified softwood cuttings had the highest survival, broadly agreeing with findings for Sichuan pepper, vine pepper, and other *Zanthoxylum* plants. Cuttings that are too short have insufficient carbohydrate and endogenous auxin reserves to support adventitious root formation. Excessive cutting length or lignification increases the transpiring surface and reduces cambial cell activity. In production, cuttings should be collected strictly from vigorous mother plants whose prickle-free trait has remained stable over multiple generations. Water shoots and branches affected by pests or diseases should be avoided, as these choices are essential for planting material quality and trait stability.

ABT rooting powder improves rooting by supplying exogenous auxin and promoting cambial cell division and root primordium differentiation at the cut surface. This study found that 100 $mg \cdot L^{-1}$ soaking for 3 h was suitable for hardwood cuttings, while 30 $mg \cdot L^{-1}$ soaking for 1.5 h was suitable for softwood cuttings, a roughly threefold difference in concentration. This difference reflects the combined effect of changes in ABT concentration and treatment duration. Hardwood cuttings have thick cortical tissues and a high degree of cutinization, slowing uptake of the agent and requiring a higher concentration and longer treatment. Softwood cuttings have tender tissues and high epidermal permeability, so high con-

centrations readily cause browning and phytotoxicity at the cut surface. This agrees with earlier observations that low-concentration NAA/IBA mixtures performed well in Sichuan pepper softwood cuttings.

The substrate provides the microenvironment for cutting rooting. Hardwood cutting propagation takes longer, with callus formation dominating the early stage, and requires a substrate with some nutrient-retention capacity. The mixture of humus-rich soil, river sand, and composted organic fertilizer therefore performed best. Softwood cuttings are planted during the hot summer season and require greater aeration. The porous river sand:perlite:vermiculite mixture (1:1:1) effectively reduced basal rot. This agrees with the importance of porosity and air-water balance reported in substrate studies of woody plant cuttings. Substrate disinfection is also important. Carbendazim drenching followed by covering and sealing the nursery under plastic was used in this study to effectively reduce damping-off and root rot.

Stable retention of the prickle-free trait is central to propagation of superior prickle-free Sichuan pepper material. Natural hybridization and introgression within *Zanthoxylum* species are common. Huyan *et al.* (2025) noted substantial trait segregation in seed-derived *Z. armatum* populations and the need for vegetative propagation and progeny testing to ensure stable inheritance of superior individuals [2]. In this study, seed-derived plants had only approximately 65% prickle-free trait retention and 35% prickle reappearance, further indicating the genetic unreliability of seed propagation for prickle-free clones. By comparison, both hardwood- and softwood-cutting-derived plants had 100% prickle-free trait retention one year after transplanting, demonstrating that cutting propagation is an effective means of stable multiplication of superior prickle-free green Sichuan pepper clones. Long-term trait stability nevertheless requires continued field observation through the fruiting stage of mature plants.

5. Conclusions

1) For hardwood cutting propagation of prickle-free green Sichuan pepper, one-year-old dormant shoots 15 - 20 cm long with 3 - 4 plump buds from 3 - 5-year-old mother plants with a stable prickle-free trait are recommended. Cuttings should be soaked in 100 mg·L^{-1} ABT rooting powder for 3 h and planted in humus-rich soil:river sand:composted organic fertilizer (2:2:1), with soil water content maintained at 60% - 70% and temperature at 20 - 25˚C. Survival reached 85.0% in the single-factor rooting-treatment experiment.

2) For softwood cutting propagation of prickle-free green Sichuan pepper, semi-lignified current-season shoots are recommended, retaining 2 - 3 upper leaves trimmed by half. Cuttings should be soaked in 30 mg·L^{-1} ABT for 1.5 h and planted in river sand:perlite:vermiculite (1:1:1). A low plastic tunnel should be used to retain humidity, with shading and cooling to maintain air relative humidity at 85% - 90%. Survival reached 82.0% in the single-factor rooting-treatment experiment.

3) Cutting propagation can stably retain the favorable prickle-free trait in green Sichuan pepper. Prickle-free trait retention was 100% one year after transplanting, significantly higher than in seed-derived plants (approximately 65%). The standardized cutting protocol established in this study can support large-scale nursery propagation of superior prickle-free green Sichuan pepper clones.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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