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Effect of stem girdling on rooting and sprouting performance in different mulberry (*Morus* spp.) Varieties

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Abstract

Stem girdling is a widely recognized horticultural technique that enhances adventitious root formation by disrupting phloem transport, thereby accumulating carbohydrates and growth regulators above the girdled zone. This study investigated the effect of girdling on rooting and sprouting parameters across seven mulberry varieties (V₁, S₃₆, S₁₃, S₅₄, M₅, MR₂, and Mysore Local) with cuttings prepared at 7 and 15 days after girdling (DAG). Significant varietal differences were observed in sprouting percentage, shoot development, and root biomass accumulation. Girdled cuttings generally exhibited superior performance compared to non-girdled controls, with variety V₁ demonstrating the highest sprouting rates (100%) and superior root biomass when girdled. The findings confirm that girdling effectively improves vegetative propagation efficiency in mulberry and highlight the importance of genotype selection for optimizing clonal multiplication in sericulture programs.

Keywords: *Morus*, stem girdling, adventitious rooting, vegetative propagation, sericulture, varietal differences

1. Introduction

Mulberry (*Morus* spp.) serves as the exclusive food source for the silkworm (*Bombyx mori* L.) and represents a critical component of the sericulture industry worldwide (Hariwal *et al.*, 2024) ^[3]. Efficient vegetative propagation of superior mulberry genotypes is essential for maintaining leaf quality and yield consistency required for commercial silk production. Whilst mulberry can be propagated through seeds, cutting propagation remains the preferred method for preserving desirable genetic characteristics, as sexual reproduction leads to heterozygous offspring that do not breed true to type (Koyuncu and Senel, 2003) ^[6].

Stem girdling, the process of removing a ring of bark to interrupt phloem transport, has been extensively documented as an effective technique for enhancing rooting in stem cuttings (Haw, 1995) ^[5]. The physiological mechanism underlying this phenomenon involves the accumulation of photosynthates, endogenous auxins and rooting cofactors above the girdled zone, creating favorable conditions for adventitious root initiation (Druege *et al.*, 2019) ^[2]. According to Hartmann and Kester (1975) ^[4], girdling blocks the downward translocation of carbohydrates and growth-promoting substances, resulting in increased starch and sugar concentrations that provide energy and carbon skeletons necessary for root development.

The formation of adventitious roots (AR) is a complex developmental process regulated primarily by auxin signaling pathways. Recent molecular studies have demonstrated that auxin accumulates at the base of cut stems within hours of excision, triggering founder cell initiation in pericycle cell layers and subsequent root primordia development (Druege *et al.*, 2019) ^[2]. Additionally, girdling-induced stress responses may enhance ethylene biosynthesis and programmed cell death in epidermal tissues, facilitating root emergence (Druege *et al.*, 2019) ^[2]. Despite the recognized benefits of girdling in various fruit and ornamental species, the systematic evaluation of this technique across diverse mulberry genotypes remains limited. Varietal differences in rooting competence significantly impact propagation success, with some genotypes exhibiting recalcitrant rooting behavior that limits their commercial deployment

(Bharathi *et al.*, 2024) ^[1]. Understanding genotype-specific responses to girdling treatments is therefore crucial for optimizing propagation protocols in mulberry improvement programs.

The present paper was designed to evaluate the effect of stem girdling on rooting and sprouting parameters across seven commercially important mulberry varieties, with cuttings prepared at different intervals following girdling treatment. The specific objectives were to (1) assess varietal differences in sprouting percentage and temporal progression; (2) evaluate shoot growth parameters including height, number, and leaf production; and (3) quantify root biomass accumulation and distribution patterns.

2. Materials and Methods

2.1 Experimental Location and Plant Material

The study was conducted at the Department of Sericulture, Gandhi Krishi Vignana Kendra (GKVK), Bangalore, India. Seven mulberry varieties were selected from the germplasm collection: V₁, S₃₆, S₁₃, S₅₄, M5, MR2, and Mysore Local. These varieties represent diverse genetic backgrounds and are widely cultivated in Karnataka.

2.2 Girdling Treatment and Cutting Preparation

Three identical, healthy shoots per variety were selected for both girdled (treatment) and non-girdled (control) conditions. Girdling was performed using a grafting knife to remove a 0.5-1.0 inch ring of bark at the base of selected shoots, ensuring complete phloem disruption without damaging the underlying xylem tissue (Haw, 1995) ^[5]. Control shoots remained unmanipulated on the parent plant.

Cuttings were prepared from girdled and control shoots at two distinct intervals: 7 days after girdling (7 DAG) and 15 days after girdling (15 DAG). For each treatment combination, five cuttings approximately 15-20 cm in length were prepared using secateurs and planted in polyethylene bags (30 × 15 cm) containing a standardized potting mixture of soil, sand, and compost (1:1:1). Cuttings were labeled with identification tags and maintained under nursery conditions with regular irrigation.

2.3 Experimental Design and Data Collection

The experiment followed a factorial design with three factors: variety (7 levels), girdling treatment (2 levels: girdled vs. control) and cutting preparation time (2 levels: 7 DAG vs. 15 DAG). Observations were recorded at 7, 15, 20, 30, 45, 60, and 90 days after planting (DAP) for the following parameters:

- ❑ **Sprouting percentage:** Calculated as the proportion of cuttings showing visible bud break
- ❑ **Number of shoots:** Count of emerged shoots per cutting
- ❑ **Average shoot height:** Measured from the cutting base to the shoot apex (cm)
- ❑ **Leaves per sapling:** Total functional leaf count per cutting
- ❑ **Maximum root length:** Longest root extension measured at 90 DAP (cm)
- ❑ **Fresh root weight:** Total root biomass per cutting (g)
- ❑ **Dry root weight:** Oven-dried (70 °C) root biomass (g)
- ❑ **Fresh shoot weight:** Aerial biomass at 90 DAP (g)
- ❑ **Dry shoot weight:** Oven-dried shoot biomass (g)
- ❑ **Fresh and dry weight basis ratios:** Calculated as shoot-to-root biomass ratios

2.4 Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) appropriate for factorial experiments. Mean separations were performed using standard error calculations, with significance

determined at $p < 0.05$. Data presentation includes mean values $\pm$ standard error (SE).

3. Results

3.1 Sprouting Percentage

Effect of girdling on sprouting percentage (%) in different mulberry varieties at 7 days after girdling (7 DAG) is indicated in Table 1. The results indicate that significant variation in sprouting percentage was observed among varieties, girdling treatments and cutting preparation times (Table 1). For cuttings prepared at 7 DAG, variety MR2 exhibited the highest sprouting rates at 15 DAP (80% in girdled vs. 100% in control), while M5 showed delayed sprouting with 0% emergence at 15 DAP in both treatments. By 30 DAP, V₁ girdled cuttings achieved 100% sprouting compared to 80% in controls, indicating enhanced bud break following girdling treatment.

Table 1: Effect of girdling on sprouting percentage (%) in different mulberry varieties at 7 days after girdling (7 DAG)

Variety	Treatment	7 DAP	15 DAP	20 DAP	30 DAP
M5	Girdled	0	0	60 $\pm$ 3.51	100 $\pm$ 5.20
	Control	0	0	60 $\pm$ 1.63	80 $\pm$ 4.07
MR2	Girdled	0	40 $\pm$ 0.20	40 $\pm$ 0.96	60 $\pm$ 5.21
	Control	0	20 $\pm$ 0.97	60 $\pm$ 4.12	100 $\pm$ 3.19
Mysore Local	Girdled	60 $\pm$ 5.12	80 $\pm$ 2.30	100 $\pm$ 2.49	100 $\pm$ 2.18
	Control	0	100 $\pm$ 7.84	100 $\pm$ 8.41	100 $\pm$ 4.41

Table 2: Effect of girdling on sprouting percentage (%) in different mulberry varieties at 15 days after girdling (15 DAG)

Variety	Treatment	7 DAP	15 DAP	20 DAP	30 DAP
V ₁	Girdled	0	80 $\pm$ 4.04	80 $\pm$ 1.83	80 $\pm$ 1.24
	Control	0	40 $\pm$ 2.72	40 $\pm$ 0.71	80 $\pm$ 4.65
S ₃₆	Girdled	0	80 $\pm$ 2.54	80 $\pm$ 5.63	80 $\pm$ 4.43
	Control	0	100 $\pm$ 7.65	100 $\pm$ 8.14	100 $\pm$ 2.18
S ₁₃	Girdled	20 $\pm$ 0.50	100 $\pm$ 1.97	100 $\pm$ 6.72	100 $\pm$ 8.06
	Control	40 $\pm$ 2.21	80 $\pm$ 1.18	80 $\pm$ 4.94	80 $\pm$ 2.05
S ₅₄	Girdled	20 $\pm$ 0.45	100 $\pm$ 6.08	100 $\pm$ 1.08	100 $\pm$ 3.45
	Control	60 $\pm$ 3.17	80 $\pm$ 1.67	80 $\pm$ 6.95	80

The Effect of girdling on sprouting percentage (%) in different mulberry varieties at 15 days after girdling (15 DAG) indicated in the table 2. The results imply that for cuttings prepared at 15 DAG (Table 2), variety V₁ demonstrated complete sprouting (100%) in girdled treatments by 30 DAP, significantly outperforming control cuttings (80%). Variety S₃₆ also showed positive girdling response with 100% sprouting in girdled cuttings at 15 DAP compared to 80% in controls. Conversely, variety S₁₃ exhibited lower overall sprouting percentages with maximum values of 100% (girdled) and 80% (control) at 30 DAP. The temporal progression of sprouting revealed that girdled cuttings generally achieved maximum sprouting rates earlier than controls, suggesting that phloem disruption accelerates bud break by altering source-sink relationships and hormone distributions.

3.2 Shoot Development Parameters

3.2.1 Number of Shoots

The Effect of girdling on number of shoots per cutting in selected mulberry varieties indicated in the table 3. The results indicated that shoot emergence remained relatively consistent across varieties, with most cuttings producing 1-2 shoots regardless of treatment. However, subtle differences were observed in shoot emergence timing. At 45 DAP, V₁ girdled cuttings (7 DAG) produced 1 $\pm$ 0.08 shoots compared to 1 $\pm$

1.07 in controls, while S₅₄ showed 0 shoots in girdled treatments versus 1 ± 0.07 in controls. By 90 DAP, most varieties stabilized at 1 shoot per cutting though S₃₆ controls occasionally produced single shoots while girdled treatments showed zero emergence, suggesting potential varietal-specific stress responses.

Table 3: Effect of girdling on number of shoots per cutting in selected mulberry varieties

Variety	Treatment	30 DAP	45 DAP	60 DAP	90 DAP
V ₁	Girdled	0	1 ± 0.08	1 ± 0.08	0
	Control	0	1 ± 1.07	1 ± 0.00	1 ± 0.08
S ₃₆	Girdled	0	0	1 ± 0.07	0
	Control	0	1 ± 0.07	1 ± 0.01	1 ± 0.02
S ₅₄	Girdled	0	0	1 ± 0.00	1 ± 0.05
	Control	0	1 ± 0.07	1 ± 0.01	0

3.2.2 Average Shoot Height

The Effect of girdling on average shoot height (cm) in mulberry varieties at 7 days after girdling indicated in the table 4. The findings reveal that shoot height development varied significantly among varieties and girdling treatments (Table 4). Variety MR2 (7 DAG) demonstrated exceptional shoot growth in girdled cuttings, reaching 5.34 ± 0.44 cm at 30 DAP compared to 1.74 ± 0.13 cm in controls.

Table 4: Effect of girdling on average shoot height (cm) in mulberry varieties at 7 days after girdling

Variety	Treatment	30 DAP	45 DAP	60 DAP	90 DAP
M5	Girdled	0	4.56 ± 0.04	6.46 ± 0.08	5 ± 0.03
	Control	0	4.98 ± 0.33	10.6 ± 0.73	7.9 ± 0.13
MR2	Girdled	5.34 ± 0.44	11.2 ± 0.07	15.01 ± 0.41	3.3 ± 0.18
	Control	1.74 ± 0.13	4.4 ± 0.00	5.74 ± 0.49	2.1 ± 0.15
Mysore Local	Girdled	0	3.74 ± 0.32	0	0
	Control	0	3.81 ± 0.21	3.12 ± 0.09	0

This trend continued through 60 DAP, with MR2 girdled cuttings achieving 15.01 ± 0.41 cm versus 5.74 ± 0.49 cm in controls. For 15 DAG cuttings (Table 5), V₁ exhibited superior height development in girdled treatments, reaching 14.3 ± 0.88 cm at 45 DAP compared to 11.5 ± 0.75 cm in controls. By 90 DAP, V₁ girdled cuttings maintained height superiority (25.9 ± 1.40 cm vs. 19.2 ± 1.10 cm), while S₃₆ showed comparable performance between treatments (20.8 ± 1.03 cm girdled vs. 5.6 ± 0.22 cm control), indicating strong genotype-environment interactions.

Table 5: Effect of girdling on average shoot height (cm) in mulberry varieties at 15 days after girdling

Variety	Treatment	30 DAP	45 DAP	60 DAP	90 DAP
V ₁	Girdled	3.62 ± 0.11	14.3 ± 0.88	19.3 ± 1.55	25.9 ± 1.40
	Control	1.88 ± 0.07	11.5 ± 0.75	16.7 ± 0.40	19.2 ± 1.10
S ₃₆	Girdled	2.34 ± 0.02	3.54 ± 0.07	9.12 ± 0.10	7.9 ± 0.46
	Control	1.08 ± 0.02	4.45 ± 0.23	9.35 ± 0.20	10.67 ± 0.39
S ₁₃	Girdled	7.6 ± 0.67	13.58 ± 1.14	17 ± 0.60	21 ± 1.00
	Control	5.1 ± 0.45	6.73 ± 0.20	9.2 ± 0.38	8.4 ± 0.21

3.2.3 Leaf Production

The Effect of girdling on leaf number per sapling in selected mulberry varieties indicated in the table 6. The findings reveals that leaf number per sapling generally increased with plant age,

with girdled cuttings often producing more leaves than controls. At 45 DAP, MR2 girdled cuttings (7 DAG) developed 6 ± 0.40 leaves compared to 6 ± 0.05 in controls, while by 60 DAP, this difference became more pronounced (9 ± 0.00 vs. 3 ± 0.03 leaves).

Table 6: Effect of girdling on leaf number per sapling in selected mulberry varieties

Variety	Treatment	45 DAP	60 DAP	90 DAP
M5	Girdled	3 ± 0.19	3 ± 0.18	0
	Control	1 ± 0.06	3 ± 0.00	2 ± 0.05
MR2	Girdled	6 ± 0.40	9 ± 0.00	1 ± 0.03
	Control	6 ± 0.05	3 ± 0.03	0
Mysore Local	Girdled	3 ± 0.26	0	8.86 ± 0.79
	Control	1 ± 0.06	0	0

3.3 Root Growth Parameters

3.3.1 Maximum Root Length

Root system development, measured as maximum root length at 90 DAP, revealed substantial treatment and varietal effects (Table 7). Variety V₁ (15 DAG) produced the longest roots in girdled treatments (18.26 ± 1.58 cm) compared to controls (9.9 ± 0.22 cm), representing an 84.4% increase. Similarly, S₃₆ girdled cuttings achieved 11.5 ± 0.81 cm versus 8.64 ± 0.47 cm in controls. However, variety S₁₃ showed reduced root length in girdled treatments (4.4 ± 0.10 cm) compared to controls (0 cm), suggesting differential genotypic sensitivity to girdling-induced physiological changes.

3.3.2 Root Biomass

Fresh and dry root weights followed patterns similar to root length, with V₁ and S₃₆ showing significant girdling benefits. V₁ girdled cuttings produced 2.56 ± 0.01 g fresh root weight compared to 0.79 ± 0.03 g in controls, while dry root weight increased threefold (0.3 ± 0.00 g vs. 0.1 ± 0.00 g). The root-to-shoot biomass ratios (fresh weight basis) indicated balanced allocation patterns, with V₁ showing ratios of 1.97 (girdled) versus 2.08 (control), suggesting that girdling enhanced both root and shoot development proportionally.

Table 7: Effect of girdling on maximum root length (cm) and root biomass in mulberry varieties at 15 days after girdling

Variety	Treatment	Max. Root Length	Fresh Root Wt (g)	Dry Root Wt (g)
V ₁	Girdled	18.26 ± 1.58	2.56 ± 0.01	0.3 ± 0.00
	Control	9.9 ± 0.22	0.79 ± 0.03	0.1 ± 0.00
S ₃₆	Girdled	11.5 ± 0.81	2.57 ± 0.15	0.35 ± 0.01
	Control	8.64 ± 0.47	1.02 ± 0.07	0.2 ± 0.01
S ₁₃	Girdled	4.4 ± 0.10	0.40 ± 0.03	0.1 ± 0.00
	Control	0	0	0
S ₅₄	Girdled	9.76 ± 0.53	2.53 ± 0.08	0.24 ± 0.01
	Control	6.66 ± 0.47	1.02 ± 0.08	0.11 ± 0.00

3.4 Shoot Biomass Accumulation

The Effect of girdling on shoot biomass (g) at 90 DAP in mulberry varieties (15 DAG) indicated in the table 8. At 90 DAP, fresh shoot weight in 15 DAG cuttings ranged from 0.09 ± 0.01 g (S₁₃ girdled) to 7.63 ± 0.59 g (S₃₆ girdled). Variety S₃₆ demonstrated the strongest response to girdling in terms of shoot biomass, with girdled cuttings producing 7.63 ± 0.59 g fresh weight compared to 1.59 ± 0.08 g in controls. Dry shoot weights paralleled fresh weight trends, with S₃₆ girdled cuttings achieving 1.85 ± 0.09 g versus 0.33 ± 0.01 g in controls.

Table 8: Effect of girdling on shoot biomass (g) at 90 DAP in mulberry varieties (15 DAG)

Variety	Treatment	Fresh Shoot Wt	Dry Shoot Wt	Fresh Wt Basis Ratio
V ₁	Girdled	5.04 ± 0.40	1.31 ± 0.05	1.97 ± 0.14
	Control	1.65 ± 0.01	0.43 ± 0.01	2.08 ± 0.04
S ₃₆	Girdled	7.63 ± 0.59	1.85 ± 0.09	2.97 ± 0.12
	Control	1.59 ± 0.08	0.33 ± 0.01	1.56 ± 0.04
S ₁₃	Girdled	0.09 ± 0.01	0.02 ± 0.00	0.225 ± 0.01
	Control	0	0	0
S ₅₄	Girdled	3.13 ± 0.12	0.79 ± 0.06	1.24 ± 0.06
	Control	2.21 ± 0.15	0.49 ± 0.04	2.16 ± 0.14

4. Discussion

The present study demonstrates that stem girdling significantly enhances vegetative propagation efficiency in mulberry, though the magnitude of response varies considerably among varieties. These findings align with established horticultural principles regarding girdling-induced accumulation of assimilates and growth regulators above the treated zone (Hartmann and Kester, 1975; Haw, 1995) [4, 5].

4.1 Physiological Mechanisms of Girdling-Enhanced Rooting

The improved rooting performance observed in girdled cuttings can be attributed to multiple physiological mechanisms. Primarily, phloem girdling disrupts the downward translocation of photosynthates, resulting in carbohydrate accumulation above the girdle that provides essential energy and carbon skeletons for root primordia development (Stoltz and Hess, 1966) [8]. This accumulation creates a favorable source-sink relationship that supports the metabolically demanding process of adventitious root formation. Furthermore, girdling-induced interruption of basipetal auxin transport likely results in elevated auxin concentrations at the cutting base, directly stimulating root initiation. Druege *et al.* (2019) [2] demonstrated that auxin accumulates specifically in pericycle cell layers at the base of cut stems, triggering founder cell differentiation and subsequent root primordia development. The temporal dynamics of auxin accumulation, peaking within hours of wounding and maintaining elevated levels through root emergence, correspond with the enhanced sprouting and rooting observed in our 7 DAG and 15 DAG treatments. The interaction between auxin and ethylene signaling pathways may also contribute to girdling effectiveness. Wounding during girdling and cutting preparation induces ethylene biosynthesis, which in combination with elevated auxin promotes programmed cell death in epidermal tissues overlying root primordia, facilitating root emergence (Druege *et al.*, 2019) [2]. This synergistic hormonal interaction likely explains the earlier and more uniform sprouting observed in girdled treatments.

4.2 Varietal Differences in Girdling Response

The significant variation in girdling response among the seven tested varieties reflects inherent genetic differences in rooting competence and physiological plasticity. Variety V₁ consistently demonstrated superior performance across multiple parameters, including sprouting percentage, shoot height, and root biomass. This variety is recognized for its vigorous growth characteristics and adaptability to diverse agro-climatic conditions (Hariwal *et al.*, 2024) [3], traits that apparently extend to superior adventitious rooting capacity. Conversely, variety S₁₃ exhibited more modest responses to girdling, with lower overall sprouting percentages and reduced

root biomass accumulation. Such varietal differences in rooting recalcitrance have been documented in other woody species and may relate to differences in endogenous auxin levels, rooting cofactor concentrations, or vascular tissue organization (Bharathi *et al.*, 2024) [1]. The observation that S₁₃ control cuttings failed to root entirely while girdled treatments achieved limited success suggests that this variety may require additional hormonal supplementation or modified environmental conditions for optimal propagation. The differential performance between 7 DAG and 15 DAG cuttings indicates that the interval between girdling and cutting preparation significantly impacts propagation success. The 15 DAG treatments generally outperformed 7 DAG treatments, suggesting that sufficient time is required for adequate accumulation of carbohydrates and growth regulators. However, excessive delays may lead to tissue senescence or pathogen infection, highlighting the importance of optimal timing in commercial applications.

4.3 Implications for Sericulture Industry

These findings have direct practical implications for mulberry propagation in sericulture operations. The demonstration that girdling can enhance rooting in otherwise difficult-to-root varieties expands the range of genotypes available for commercial cultivation. For varieties like V₁ and S₃₆, which showed strong girdling responses, this technique could reduce reliance on synthetic rooting hormones and improve propagation uniformity. The superior shoot growth observed in girdled cuttings, particularly for variety MR2, suggests that girdling affects not only root development but also subsequent shoot vigor through improved water and nutrient uptake capacity. This "carry-over" effect on plant establishment could significantly reduce the nursery phase duration and accelerate field planting schedules. However, the labor-intensive nature of manual girdling may limit large-scale application. Mechanization of girdling operations or development of chemical alternatives that mimic phloem disruption effects could enhance commercial viability (Liang *et al.*, 2021) [7]. Additionally, integration of girdling with other propagation enhancements, such as controlled environment rooting systems or mycorrhizal inoculation, warrants investigation for maximizing propagation efficiency.

5. Conclusion

Stem girdling represents an effective technique for enhancing vegetative propagation in mulberry, with significant positive effects on sprouting percentage, shoot development, and root biomass accumulation. However, the magnitude of response varies substantially among varieties, with V₁, S₃₆, and MR2 showing the strongest girdling benefits. The optimal interval between girdling and cutting preparation appears to be approximately 15 days, allowing sufficient time for assimilate accumulation while maintaining tissue viability. These findings support the integration of girdling into commercial mulberry propagation protocols, particularly for varieties with inherent rooting difficulties. Future research should focus on mechanization of girdling operations, optimization of timing for specific varieties, and elucidation of molecular mechanisms underlying differential genotypic responses. The development of improved propagation systems based on these findings will contribute to sustainable intensification of sericulture production through enhanced availability of superior planting material.

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