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The influence of substrate and cuttings' type on rooting capacity of *Morus alba* species

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Abstract
The cultivation of white mulberry has a long and important history being an important crop for sericulture but also for humans due to its various therapeutic properties. The number of mulberry trees as food plants for the sericulture industry significantly decreased in the last years. Therefore, the main aim of this research was to optimize mulberry propagation methods by hardwood cuttings, since its propagation by seeds is undesirable due to cross-pollination and pronounced heterozygosity of the plants. Besides, mulberry seeds are photosensitive and requires high temperatures to germinate (min. 8 light hours and 30 °C during the day versus 16 hours of 20 °C during night). In order to break seed dormancy pretreatments are needed which can take up to 8-9 weeks and are expensive. To carry out this study two types of cuttings (straight and mallet), 4 different rooting medium mixtures and a rooting hormone were used to test the efficiency of the method. The results show that mallet cuttings treated with radi-stim rooting hormone and inserted into a mixture of universal substrate and sand reached the highest rooting percentage as compared to the other experimental treatments.

Keywords: hardwood cuttings, white mulberry, propagation, rooting medium

Introduction

The mulberry genus, scientifically known as *Morus* comprises about 100 species which are native to various regions from temperate to subtropical regions of Asia, Africa, Europe, North and South- America. Among the 100 species, *Morus alba* L. (white mulberry), *Morus nigra* L. (black mulberry), *Morus rubra* L. (red mulberry) and *Morus indica* L. are the most commonly known all over the world [1, 2, 7]. 24 species of originate from China and 19 species from Japan [6, 9]. All members of this genus are fast growing, deciduous fruit trees with a great economic importance for their multiple uses. Since ancient times, mulberry leaves were the main source of animal fodder providing the primary food for silkworms being vital for the silk industry. The wood of mulberry has also been used for timber and subsequently for making furniture or sports items. The bark of paper mulberry, *Morus papyrifera* L., in some countries named as *Broussonetia papyrifera* was used for barkcloth and high-quality paper production [26, 30]. Nowadays, the wood of the plant is suitable for making furniture and utensils, while its fruits and cooked leaves are edible. Mulberry fruits are important food sources for humans as well due to their enhanced therapeutic properties as follow: antioxidant properties, inhibiting LDL oxidation, neurodegenerative disorder and mode of action in stimulating skin tone [20]. Numerous studies have been carried out to confirm their chemical profile. They are rich in proteins, phenols, flavonoids, and anthocyanins that enhance their significance as nature's promising functional tonic [31]. Mulberry leaves contain antimicrobial agents, while mulberry extracts or components, especially flavonoids (quercetin, rutin, and isoquercitrin) scavenge free radicals that show potential against oxidative stress.

The cultivation of mulberry trees in Transylvania, from Romania dates back to the 15th century along the sericulture industry. In 1944 the annual cocoon production was 500 tons and was increasing through the upcoming years over 1894 tons in 1989 [22, 24]. After this period, a drastic drop was recorded when the cocoon's production was diminished to 42 tons in 1993. Despite the decrease in cocoon's production, the

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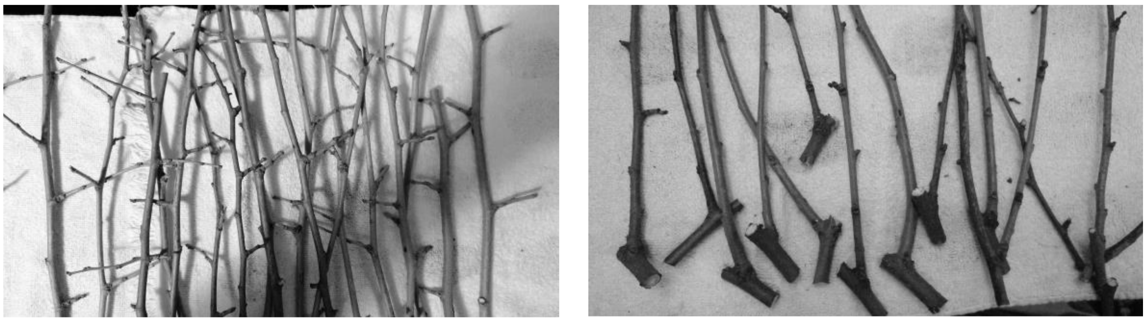
favorable climatic conditions, and sericulture tradition of Romania strengthen the potential of the country to regain its power towards mulberry cultivation and sericulture. According to a study carried out by Hăbeanu et al., 2023, growing silkworms increased steadily until the beginning of the 21st century [8, 10].

In this context, the high-quality planting material production for mulberry plantations remains a challenge. Mulberry trees can be propagated through various methods such as seeds, cuttings, grafting methods or in vitro cultures but some methods are less successful but costly. Mulberry propagation by seeds is undesirable for high-quality planting material production due to cross-pollination and pronounced heterozygosity of the plants [12, 14]. Besides, seeds require special germinating conditions or pretreatments due to their photo- and temperature-sensitive character. The reproduction of mulberry plants by in vitro culture could be a great alternative, but it takes time and requires specific laboratory equipment and high costs. In this regard, the propagation of mulberry plants through hard wood cuttings remains a short-time, high uniformity and low-cost technique which is applied for commercial propagation as well, but further research are still needed to improve the rooting capacity of the cuttings [27]. Previous studies show that the time of mulberry cuttings' s preparation highly affects root formation. Similarly, it was found that the physiological conditions of the plants and environmental conditions have a great impact of the success of reproduction. Other factors are also known which could affect the rooting capacity of the cuttings such as cultivar type, the age of the mother plant and shoots, position and type of cuttings prepared, wounding or leaf removal and growing conditions including rooting media, mist, bottom heat, the use of rooting hormones and fertilizers and extra lighting [15]. Taking into consideration the aforementioned difficulties, the main aim of the current research was to investigate the influence of hard wood cutting types and rooting media composition supplemented with rooting hormone on the root formation in *Morus alba* L.

Material and Method

Preparation of the cuttings

White mulberry shoots were collected in the 15th of February 2023 from mature and healthy trees belonging to the local white mulberry population of Deva city (45°52′N; 22°54′E), Romania. From the collected shoots straight and mallet cuttings were further prepared with the help of a sterilized pruning shear. For straight stem cuttings 1/3 from both of the upper and bottom part of the shoots were eliminated, and the middle part of every shoot was used for straight cutting preparation. 3-4-nodes long and 6-8 mm Ø cuttings were prepared and inserted into various rooting substrates (Figure 1a). The top of the cuttings was slanted at 1 cm above the last node while the bottom part had a perpendicular cut to provoke the rooting. For the preparation of mallet cuttings, a section about 1 cm in length was taken from the last year's growth which had a sideshoot of the current season on it and cut just above and below the side shoot (Figure 1b).



(a) (b)

Figure 1. Straight (a) and mallet (b) white mulberry cuttings prepared for propagation

Rooting mediums

To determine the influence of substrate on rooting capacity and growth, 5 different potting soils were used as rooting media components as follow:

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Potting soil type		Specifications
1. Universal potting mixture		pH 7.0; N>0.3 (m/m%), P2O5 >0.1 (m/m%), K2O>0.1 (m/m%) and organic matter: 50 (m/m%).
2. Compressed Coconut fiber		30% coconut husk pieces; 70% coconut fiber. pH 5,6-6,8. Water holding capacity: 70%
3. Perlite		inorganic pH 6,5-7,5; 2-5 mm
4. Horticultural sand		Smooth and less gritty
5. Vermiculite		mica-like mineral that serves as a soil amendment containing NH_4^+, K, Mg and Ca; pH 7-7.5, Water holding capacity up to 70%

To establish the experimental design eight potting mixtures were prepared and used to root the white mulberry cuttings, which were planted in 8x8x7 cm and 10x7.5 cm plastic pots filled up with the potting mixtures and considered experimental treatments (Fig. 2). Radi-stim as a rooting hormone was also used to check its efficiency on rooting. The cuttings were maintained in greenhouse conditions and watered on a regular-basis, once a week.

Figure 2. Working steps of cutting propagation of *Morus alba* L.

Experimental design

The experimental design consisted of 8 treatments to which both types of cuttings were subjected and applied as a factorial experiment according to randomized complete block design in three replicates as follow:

1. Universal potting mix – considered as Control
2. Universal potting mix + Radi-stim
3. Compressed Coconut fiber + Perlite (2:1)
4. Compressed Coconut fiber + Perlite + Radi-stim (2:1)
5. Horticultural Sand + Universal potting mixture (1:1)
6. Horticultural Sand + Universal potting mixture (1:1) + Radi-stim
7. Vermiculite + Compressed Coconut fiber (1:3)
8. Vermiculite + Compressed Coconut fiber (1:3) + Radi-stim

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Every treatment was applied to 25 pots in three replicates containing one cutting each. During the treatments, data collection was carried out at 7-days interval. Observations were made upon the time and bud stage during the rooting period (Fig.3), followed by the quantification of root number and the measurement of the primary root length. The rooting success was determined by calculating the rooting percentage of the cuttings at the end of the rooting period. The number of primary roots arising from the base of the cuttings were counted and averaged. The length of primary roots of the rooted cuttings were measured with from the bottom of the root tip and averaged within every treatment.

(a) (b) (c)

Figure 3. Different bud stages (a, b) and sprouting (c) of *Morus alba* L. cuttings

Statistical analyses

The data presented in this study are mean values. The effects of the applied treatments on rooting success were evaluated by the Analysis of Variance (ANOVA) followed by Tukey's HSD test in order to determine statistically significant differences between the means at $p<0.05$. Data processing and statistical analyses were performed using SPSS V19 Software.

Results and Discussion

Contrary to its economic importance especially for the sericulture industry, the vegetative propagation of white mulberry through cuttings has been fallen into oblivion and ignored due to their poor rooting ability which repeatedly led to unsatisfactory results [3, 4]. Root grafting might be a practicable alternative; however, it takes up to five years to obtain the new plant. Likewise other grafting methods, root grafting requires special attention and grafting skills associated with increased costs since an apparently insignificant error in the grafting process can result in complete failure [19]. In regard to the existing constraints of mulberry propagation, the results of this research may offer an alternative or starting point for further investigations.

However, our results are consistent with previous findings which highlight the sustained and low percentage of rooting in white mulberry plants. The maximum rooting percentage recorded during this research was 40% (Fig.4). This percentage is relatively high as compared to other maximum rooting percentages reported in white mulberry reaching up to 50-60% [13]. Interestingly the lowest rooting percentage (8%) was registered for straight cuttings planted in compressed coconut fiber and perlite potting soil. The results of this research shed more light on the influence of cutting type on rooting. It can be observed that regardless the potting soils used in this experiment, mallet cuttings had a significantly higher rate of rooting as compared to straight cuttings. No statistically significant differences were found considering rooting percentage of the cuttings when those were planted into a potting mixture consisted of compressed coconut fiber+perlite+radi-stim at a significance level of $p<0.05$ according to Tukey's HSD test. However, it can also be observed that the biostimulator used increased the rooting capacity of the cuttings both for straight and mallet cuttings on the same potting soil. When no radi-stim was applied, it can be observed that the higher rooting percentage could be attributed to the cutting type as shown in Fig. 4. It is worth to be mentioned that the highest rooting percentages of the white mulberry cuttings were recorded when these were planted in universal potting mixtures with sand.

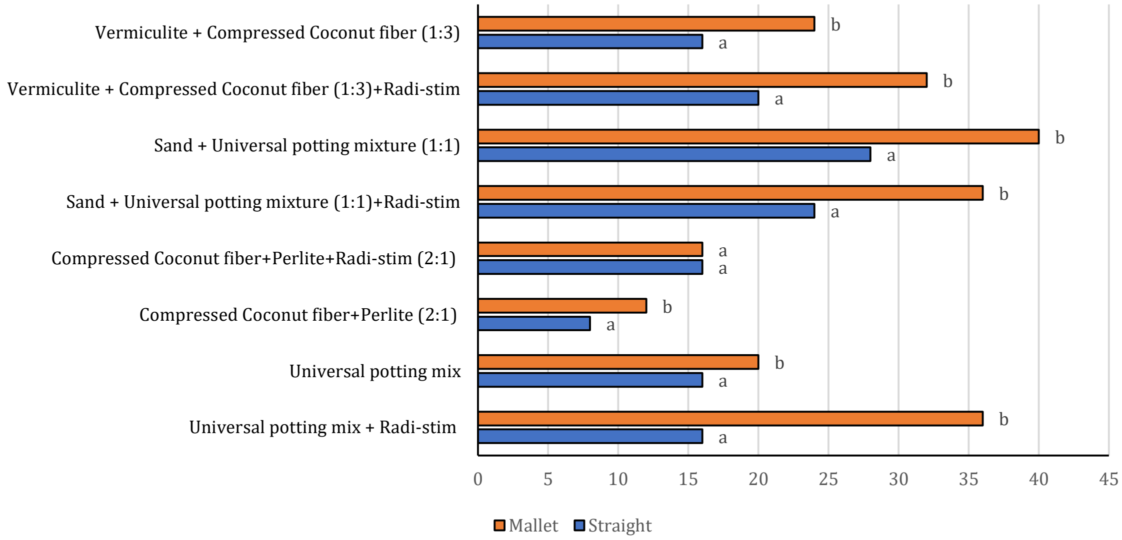

Figure 4. Rooting percentage of *Morus alba* L. cuttings on different potting soils

In general, under optimum environmental conditions, some species can generate roots in a few days, while others may take to two or three months [1, 2, 21]. The rooting period of the white mulberry cuttings lasted up to 80 days (Table 1). The results of this study show that the first swollen buds were observed mostly on mallet cuttings first in the potting soils tested. This phenomenon might be explained by the fact that callus production was facilitated earlier in mallet cuttings due to the larger surface area which has got in contact with the soil. Hereby, the absorption of the nutrient was stimulated which resulted in earlier bud swell and sprouting [17]. The rooting success of mallet and heel cuttings might also be explained by the production of root primordial in the older stems which remain dormant until the stem gets in contact with the soil and moisture or until the stem is detached from the mother plant and placed in a rooting media [17, 30]. Similar reactions have been reported in peach cuttings when out of the five types of cuttings (apical, sub-apical, basal, heel and mallet) mallet cuttings rooted first and had the greatest rooting and sprouting performance [14, 16]. The first swollen bud has been observed after 37 days on Vermiculite + Compressed Coconut fiber (1:3) potting mixture on mallet cuttings. After 57 days, other swollen buds were observed on straight cuttings as well on compressed coconut fiber + Perlite + Radi-stim (2:1) potting soil.

Table 1. Number of days until bud swell

		No. of days to bud swell	
	Potting soil/Cutting type	**Straight**	**Mallet**
1	Universal potting mix – considered as Control	52	62
2	Universal potting mix + Radi-stim	57	52
3	Compressed Coconut fiber + Perlite (2:1)	52	52
4	Compressed Coconut fiber + Perlite + Radi-stim (2:1)	52	66
5	Horticultural Sand + Universal potting mixture (1:1)	75	62
6	Horticultural Sand + Universal potting mixture (1:1) + Radi-stim	66	52
7	Vermiculite + Compressed Coconut fiber (1:3)	57	37
8	Vermiculite + Compressed Coconut fiber (1:3) + Radi-stim	62	66

At the end of the rooting period (after 90 days) the cuttings were removed from the pots and the number of primary roots were counted followed by the measurement of the primary root length. The data presented in Table 2 reveal significant differences between the treatments within the same type of cuttings regarding the number of primary roots. Thus, it can be seen that mallet cutting produced a higher number of roots that straight cuttings in all the potting soils applied. The average number of primary roots recorded in straight cuttings varied between 2.10 and 4.52. The highest number of roots were observed on cuttings planted in horticultural sand

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and universal potting mixture without and with biostimulator. The same pattern has been followed in mallet cuttings as well generating the highest primary root numbers on the same potting soil mixtures (Horticultural Sand + Universal potting mixture (1:1): primary root number=5.74; Horticultural Sand + Universal potting mixture (1:1) + Radi-stim: primary root number=5.34).

Table 2. Number of primary roots produced by the white mulberry cuttings

		No. of primary roots	
	Potting soil/Cutting type	**Straight**	**Mallet**
1	Universal potting mix – considered as Control	3.55 [bc]	4.35 [c]
2	Universal potting mix + Radi-stim	3.55 [bc]	5.33 [d]
3	Compressed Coconut fiber + Perlite (2:1)	2.10 [a]	3.17 [b]
4	Compressed Coconut fiber + Perlite + Radi-stim (2:1)	2.41 [a]	2.66 [a]
5	Horticultural Sand + Universal potting mixture (1:1)	4.52 [d]	5.75 [d]
6	Horticultural Sand + Universal potting mixture (1:1) + Radi-stim	4.22 [d]	5.34 [d]
7	Vermiculite + Compressed Coconut fiber (1:3)	3.21 [b]	4.11 [c]
8	Vermiculite + Compressed Coconut fiber (1:3) + Radi-stim	3.83 [c]	4.45 [c]

*Note: Different lowercase letter indicate statistically significant differences between the means according to Tukey's HSD test at $p<0.05$; n=25.

Data recorded on primary root length under the influence of potting soils and type of cuttings are presented in Table 3. The average primary root length of straight cuttings was ranging between 6.33 and 9.72 cm under the influence of the potting soils, while the primary root length of mallet cuttings varied from 8.44 and 11.02. For both types of cuttings, the results suggest that the potting soil influenced the growth of the primary root. The statistical analyses reveal significant differences between the means of different potting soils. It can be observed that the longest roots were developed on potting soils containing universal potting mix and sand. Similar to these, but statistically significant values were recorded in cuttings planted in coconut fiber and treated with radi-stim. Our results appear to be similar to those reported by Peer (2002) and Hartmann (1997) who tested various treatments and tried to optimize the propagation methods by cuttings in mulberry [11, 23]. Other reports suggest that auxins exert the greatest effect on rooting of the cuttings encouraging root formation, increasing thus the number of roots and their length in several mulberry varieties [5, 25, 28, 29, 18].

Table 3. Primary root length under the influence of potting soils and type of cuttings

		Primary root length (cm)	
	Potting soil/Cutting type	**Straight**	**Mallet**
1	Universal potting mix – considered as Control	6.93 [b]	10.14 [cd]
2	Universal potting mix + Radi-stim	6.53 [ab]	10.40 [d]
3	Compressed Coconut fiber + Perlite (2:1)	9.72 [d]	8.44 [a]
4	Compressed Coconut fiber + Perlite + Radi-stim (2:1)	8.75 [c]	10.00 [c]
5	Horticultural Sand + Universal potting mixture (1:1)	9.45 [d]	11.02 [e]
6	Horticultural Sand + Universal potting mixture (1:1) + Radi-stim	8.88 [c]	9.93 [c]
7	Vermiculite + Compressed Coconut fiber (1:3)	6.33 [a]	9.20 [b]
8	Vermiculite + Compressed Coconut fiber (1:3) + Radi-stim	8.33 [c]	9.45 [b]

*Note: Different lowercase letter indicate statistically significant differences between the means according to Tukey's HSD test at $p<0.05$; n=25.

Conclusions

Based on the results obtained in this research, it can be concluded that both factors such as cutting type and rooting medium had a strong effect on the performance of white mulberry cuttings. Satisfactory results were achieved by mallet cuttings which generated longer roots and a higher rooting percentage. Therefore, from the current research it can be concluded that the use of mallet stem cuttings for white mulberry propagation could be an alternative for planting material production, however further research is needed to improve and overcome propagation difficulties of this species.

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