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Original Article

Effect of Activated Charcoal on *in Vitro* Propagation of *Lythrum salicaria* L. (Lythraceae)

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ABSTRACT

Lythrum salicaria L., commonly referred to as Purple Loosestrife, is a medicinal plant that has been valued for its therapeutic properties for centuries. The aim of this study is to determine the effect of using activated charcoal on establishing the *in vitro* propagation of *Lythrum Salicaria* L. In this study, shoot explants (1.5 cm) were excised from buds that were 30 days old with a sterile scalpel blade and then cultured on full-strength MS medium supplemented with different concentrations of activated charcoal to multiply and increase the length of shoots. Additionally, to investigate the *in vitro* rooting response, shoot tip explants were cut from elongated shoots and cultured on an MS medium containing different concentrations of NAA with or without activated charcoal with three replicates in each experiment. MS medium with 0.5 g/L activated charcoal had the highest mean shoot length (7.1 cm ± 0.15) and mean number of shoots per explant (2.4±0.11). The results show that 0.5 mg/L NAA and 0.2 g/L activated charcoal provide the best response for rooting. The improved protocol can be utilized to grow roots in micro shoots of *L. salicaria*, which is an important stage in the micro propagation of *L. salicaria*.

Keyword: Activated Charcoal, *Lythrum salicaria* L, Micro propagation, Shoot, Rooting.

INTRODUCTION

Lythrum salicaria L. is a perennial herbaceous plant in the *Lythraceae* family [1]. This plant has received considerable attention due to its medicinal benefits and phytoremediation potential [2, 3]. Numerous bioactive substances, like polyphenols and other chemicals with anti-inflammatory, antioxidant, and antibacterial effects, are present in them [4]. Previous studies have confirmed the medicinal properties of *Lythrum salicaria*, including its anti-inflammatory, antioxidant, and antimicrobial effects [1], can contribute to cancer prevention [5]. Additionally, *L. salicaria* has shown promise in the treatment of skin and mucosal illnesses in traditional medicine, maybe as a result of its antibacterial action [4]. The industrial sector has a growing need for biomass because of this plant's medicinal properties and the rise in the popularity of natural medicines. Plant tissue culture is a reliable method for the production of industrial phytochemicals and can help to preserve plant species [6]. However, the micropropagation of medicinal plants may be hampered by the presence of phenolic chemicals [7]. In several studies, it has been demonstrated that activated charcoal (AC) has a favourable effect on seed germination, shoot growth, and root growth, and is widely employed in plant tissue culture to buffer the harmful effects of phenolic chemicals [8].

AC can adsorb inhibitory chemicals, address issues like phenolic browning and toxicity, and promote secondary metabolite synthesis, nitrogen absorption, and plant growth. Therefore, optimizing culture methods with activated carbon is crucial for sustainable plant propagation. Numerous studies on the effects of AC on several facets of *in vitr* culture have highlighted this substance's ability to improve plant growth, development, and micropropagation success rates [12–16]. In research on *Rosa centifolia*, Akhtar *et al*. [11] found that the browning and mortality of explants were dramatically decreased when activated carbon was added to the culture media. Also, activated carbon supplementation showed helpful effects on adventitious shoot development and subsequent plantlet survival rates in research by Poniewozik *et al*. [15] on *Paphiopedilum insigne.*

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In addition, activated carbon has demonstrated potential in enhancing the generation of secondary metabolites. When activated carbon was added to a *Catharanthus roseus* plant, Khataee *et al*. [10] noticed an increase in the synthesis of vincristine, a useful secondary metabolite having therapeutic effects. The researchers argued that AC may promote the expression of essential enzymes involved in the creation of secondary metabolites, resulting in increased output.

The aim of this research was to determine the effect of the use of activated charcoal on stablish the *in vitro* propagation of *Lythrum salicaria* L. To the best of our knowledge, the present study is first report of the micro propagation of *L. salicaria* using activated carbon.

MATERIAL AND METHODS

To collect seeds of *L. salicaria*, a random sampling method was employed in the vicinity of Lavij River, Mazandaran Province, Iran, in 2021. The collected seeds were then sent to the Research Institute of Medicinal and Medicinal Plants of Shahid Beheshti University for identification. The method of plant determination involves taxonomic expertise, morphological analysis, and comparison with existing botanical references, as well as expert identification. As part of their preparation, the seeds underwent a natural air-drying process.

Seeds sterilization and germination

For the germination of *L. salicaria* seeds, three samples were used. Each sample was rinsed with distilled water and subjected to benomyl fungicide 0.1%, 70% ethanol for 30 seconds, and sodium hydroxide 1% for 10 minutes. Subsequently, the seeds were placed in Murashige and Skoog [17] medium containing vitamins, 3% sucrose, and 0.7% agar. The pH of the medium was adjusted to 5.8 using one molar hydrochloric acid or sodium hydroxide before autoclaving at 121 °C for 15 minutes. The prepared culture medium was kept in a growth room at a temperature of 25 ± 2 °C with a photoperiod of 16 hours of light and 8 hours of darkness. This entire process was repeated three times using five separate seed samples to ensure reliable and replicable results.

Effects of Activated Charcoal on Shoot Proliferation

Using a sterile scalpel blade, shoot explants (1.5 cm in length) were removed from 30-day-old buds to study the impact of activated carbon on shoot proliferation. After that, the explants were grown in a modified Murashige and Skoog (MS) medium that had different amounts of activated carbon added. In this investigation, concentrations of 0 g/l (control), 0.2 g/l, 0.5 g/l, 0.75 g/l, 1 g/l, 1.5 g/l, and 2 g/l were investigated. For appropriate integration, the activated carbon was added to the medium before it was autoclaved.

The number of shoots per explant, the length of the shoots (in centimetres), and the number of leaves per shoot were all measured after 30 days of cultivation. To achieve accurate and repeatable findings, the experiment was conducted three times. To ascertain the significance of the observed variations between the treatment groups and statistical analysis was done.

Effects of Activated Charcoal on Rooting of Micro shoots

To investigate the effects of activated charcoal on rooting, shoot tip explants (2-3 cm) were excised from elongated shoots and placed in Murashige and Skoog (MS) media with varying concentrations of NAA (0 mg/L, 0.2 mg/L, 0.5 mg/L, 1 mg/L, 1.5 mg/L, and 2 mg/L). One set of media included NAA alone, whereas the other set had NAA and 0.2 g/L of activated charcoal.

The explants were cultured in these media under controlled laboratory conditions. Regular observations were made to monitor root formation and growth. At the end of the experiment, root length was measured using a ruler, and root weight was determined using a precision balance.

Comparing the results between different NAA concentrations and the presence or absence of activated charcoal allowed for evaluating the effects of activated charcoal on rooting. The data collected from this study provide insights into the role of activated charcoal in promoting or inhibiting root development and contribute to optimizing the rooting process in plant propagation techniques.

Acclimatization of tissue cultured plantlets

After successfully rooting the seedlings, they were carefully removed from the culture containers, and the delicate roots were gently rinsed with tap water to ensure the removal of any traces of the growth medium. The rooted seedlings were then transferred to plastic pots filled with a prepared soil composition consisting of autoclaved cocopeat, perlite, and vermicomposting in precise ratios of 50%, 25%, and 25%, respectively and placed in a greenhouse. The greenhouse provided a controlled environment with a temperature range of 27-25 °C (day/night) and a photoperiod of 16/8 hours light/dark.

To create an optimal microclimate for their adaptation, the pots were covered with nylon, serving to retain essential moisture. Over the following week, a gradual process of acclimatization took place. By gradually opening the covers of the pots, the tissue culture plants were able to adjust gradually to the external conditions while still receiving the necessary protection.

Statistical Analysis

The collected data were subjected to statistical analysis using the Statistical Package for Social Sciences (SPSS). The means and standard errors were calculated based on three replicates of each treatment. To assess the significance of the results among different treatments, a one-way analysis of variance (ANOVA) was conducted. Subsequently, Duncan's multiple range tests were performed to determine any significant differences ($P<0.05$) between the treatments. This rigorous statistical analysis allowed for robust interpretation of the data and identification of any significant variations in the experimental outcomes.

RESULTS

In Fig. 1, the germination process of *L. salicaria* seeds on the MS medium enriched with vitamins, 3% sucrose, and 0.7% agar is visually depicted. The results illustrate the remarkable germination potential of *L. salicaria* under these specific growth conditions. Within the observed timeframe of 10 days, a significant proportion of the *L. salicaria* seeds successfully sprouted (Fig. 1a). with approximately 80 % of the seeds demonstrating signs of germination. This high germination rate serves as a testament to the efficacy of the MS medium formulation and highlights the importance of the supplemented nutrients in facilitating seed viability and early growth.

This example of successful germination in *L. salicaria* seeds on the optimized MS medium underscores the importance of tailoring growth conditions to enhance seedling establishment. The combination of vitamins, sucrose, and agar in the MS medium played a vital role in providing the necessary nutrients, energy, and physical support for the germination process. These findings contribute to our understanding of optimal germination techniques and guide future efforts in promoting successful seedling establishment for *L. salicaria* and other plant species with similar growth requirements.

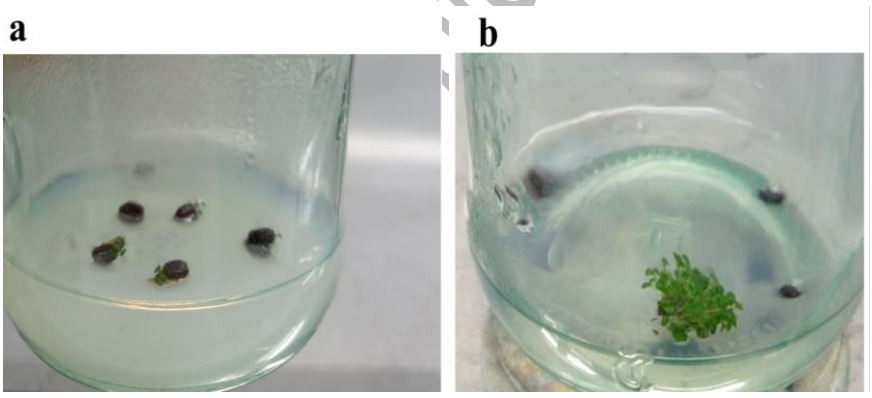

Fig. 1 Germination of *L. salicaria* seeds in MS culture: a- Germination of *L. salicaria* seeds after 10 days. b- Germination of *L. salicaria* seeds after 30 days

Shoot Proliferation

In the study of the effects of different concentrations of activated charcoal on the increase in the length of shoots of *L. salicaria*, several treatments were evaluated. The data revealed that the treatment with 0.5 g/L activated

charcoal exhibited the highest mean shoot length, measuring approximately 7.1 cm ±0.15. Additionally, this treatment resulted in an average of 2.4±0.11 shoots per sample. These findings, as presented in Table 1, indicate a significant positive effect of activated charcoal on the growth and development of *L. salicaria* shoots.

Table 1 Effects of different concentrations of activated charcoal on shoot growth of L. salicaria

Activated Charcoal (g/L)	Shoot Length (cm)	Number of Shoots per Explant	Number of Leaves per Shoot
0	2.4±0.08 a	1±0.0 a	5.2±0.11 a
0.2	4.2±0.17 b	1.6±0.0 bc	8.3±0.19 b
0.5	7.1±0.15 e	2.4±0.11 d	12.5±0.29 d
0.75	6.1±0.27 d	1.8±0.11 c	15.2±0.29 e
1	5.2±0.31 c	1.6±0.33 bc	9.3±0.50 c
1. 5	3.8±0.23 b	1.4±0.11 abc	7.5±0.29 b
2	2.8±0.23 a	1.2±0.20 ab	5.4±0.22 a

*Averages with the same letter in each column, did not different significantly between treatments by Duncan's multiple range test at $p < 0.05$.

The culture medium without activated charcoal, represented by the MS medium, demonstrated the least favourable results in terms of shoot length and number (Table 1). The minimum mean shoot length obtained from this treatment was approximately 2.4 cm ±0.08. Moreover, only one shoot per sample, with no significant variation, was observed under this condition. These results, also shown in Table 1, suggest that the absence of activated charcoal in the culture medium negatively impacted the growth and proliferation of *L. salicaria* shoots. To visually illustrate the effectiveness of the 0.5 g/L activated charcoal treatment, Fig. 2b was provided, depicting the successful establishment of *L. salicaria* seedlings. The image showcases healthy and robust seedlings that were grown using the aforementioned treatment, further supporting the superiority of this concentration of activated charcoal. Taken together, these findings underscore the crucial role of activated charcoal in promoting shoot elongation and the generation of multiple shoots in *L. salicaria*. The results emphasize the potential of 0.5 g/L activated charcoal as an effective supplement for optimizing the growth conditions of *L. salicaria* in various horticultural and research applications.

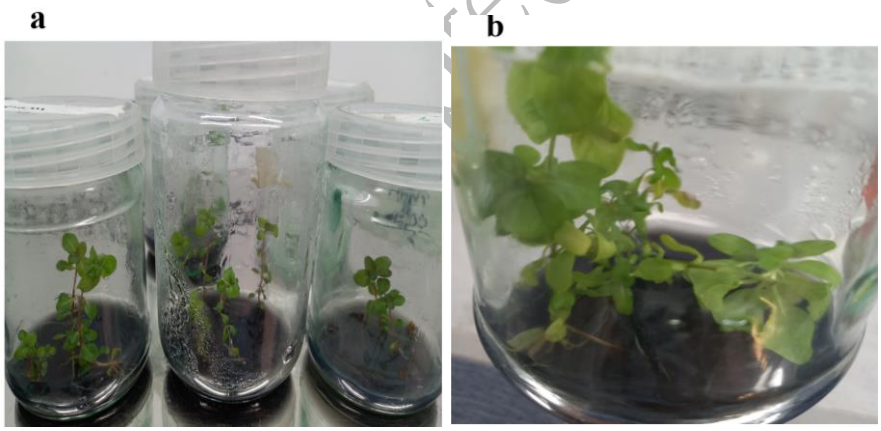

Fig. 2 Establishment of *L. salicaria* seedlings at the end of the eight weeks. a- Seedlings cultured on MS containing activated charcoal; b- Seedlings cultured on MS medium containing 0.5 g/L AC

In addition to the significant impact of activated charcoal on shoot length and the number of shoots in *L. salicaria*, further analysis was conducted to evaluate its influence on leaf growth. The results revealed an interesting relationship between activated charcoal concentration and the number of leaves produced by *L. salicaria*.

Among the different concentrations tested, it was observed that the culture medium supplemented with MS+0.75 g/L activated charcoal exhibited the maximum number of leaves (Table 1). The mean number of leaves in this treatment was approximately 15.2 ± 0.29. This outcome, as presented in Table 1, indicates a positive and stimulatory effect of activated charcoal on leaf growth in *L. salicaria*. In contrast, the control group, which did not receive any activated charcoal supplementation, displayed a lower average number of leaves. Specifically, the mean number of leaves in the control group was 5.2±0.11. These results further emphasize the importance of activated charcoal in promoting leaf development in *L. salicaria*.

However, it is worth noting that at higher concentrations of activated charcoal (equal to or greater than 1 g/L), a noticeable decrease in the number of leaves was observed. This finding, possibly attributed to the inhibitory effect of excessive activated charcoal, suggests a dose-dependent response in the leaf growth of *L. salicaria*. Further investigations may be warranted to explore the underlying mechanisms behind this observation and to determine the optimal concentration range of activated charcoal for promoting leaf growth without negative consequences. Overall, the findings from this study not only demonstrate the positive influence of activated charcoal on shoot length and shoot number but also highlight its potential in enhancing leaf growth in *L. salicaria*. The results underscore the importance of carefully selecting the appropriate concentration of activated charcoal to achieve desired growth outcomes in different plant tissues, ensuring that the benefits are maximized while avoiding any potential adverse effects.

Rooting of Micro shoots

The study also investigated the rooting of micro shoots in different culture conditions, specifically examining the effect of plant growth regulators and activated charcoal on root development in *L. salicaria*. The results yielded valuable insights into the root formation and growth characteristics of the plant.

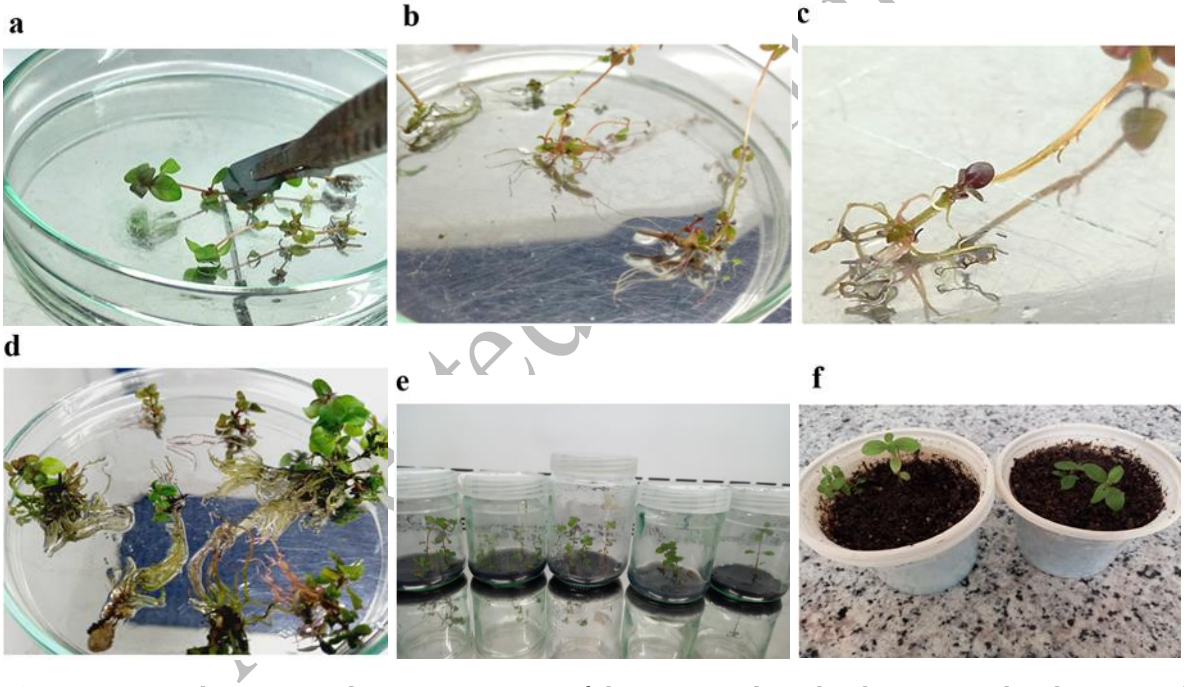

Fig. 3 Rooting in shoot apex explants. a- Preparation of shoot apex explants; b- Thin roots produced on MS medium without NAA and AC; c- The root growth on MS medium containing 0.5 mg/L NAA; d- The roots produced on MS medium containing 0.5 mg/L NAA and 0.2 g/l AC. e - Seedlings cultured on MS medium containing 0.5 mg/L NAA and 0.2 g/l AC. f- Acclimatization of the plantlets on soil after two weeks.

In the culture medium containing 0.5 mg/L NAA (Naphthaleneacetic acid) combined with 0.2 g/L activated charcoal, the percentage of rooted shoots reached an impressive 100%. This finding, as depicted in Fig.3d, suggests that the combination of NAA and activated charcoal greatly enhances the rooting process in *L. salicaria* micro shoots. In contrast, the control treatment, lacked both the plant growth regulator and activated charcoal, resulting in the production of only a few thin roots, as illustrated in Fig. 3b. This stark contrast highlights the

crucial role of NAA and activated charcoal in promoting robust root development. Furthermore, when the culture medium contained 0.5 mg/L NAA without activated charcoal, thick roots were observed (Fig. 3c). This indicates that NAA alone can induce root formation in *L. salicaria*, albeit with a different root morphology compared to the combination treatment with activated charcoal. The additional analysis focused on root length and weight further elucidated the impact of different culture conditions on root growth in *L. salicaria*. The culture medium containing 0.5 mg/L NAA and 0.2 g/L activated charcoal exhibited the maximum root length, measuring approximately 8.3 cm ± 0.08 (Fig. 4c). Similarly, the highest weight of roots, with an average of 0.72 g ± 0.01, was obtained in the same culture medium (Fig. 4d). These results, presented in Table 4, underscore the significant role of both NAA and activated charcoal in promoting root elongation and biomass accumulation in *L. salicaria*. Conversely, the culture medium without NAA and activated charcoal demonstrated the minimum root length, measuring approximately 1.3 cm ± 0.05 (Fig.4a). In addition, the lowest weight of roots, averaging 0.11 g ± 0.00, was observed in this medium (Fig. 4b). These findings emphasize the necessity of NAA and activated charcoal for facilitating optimal root growth in *L. salicaria*.

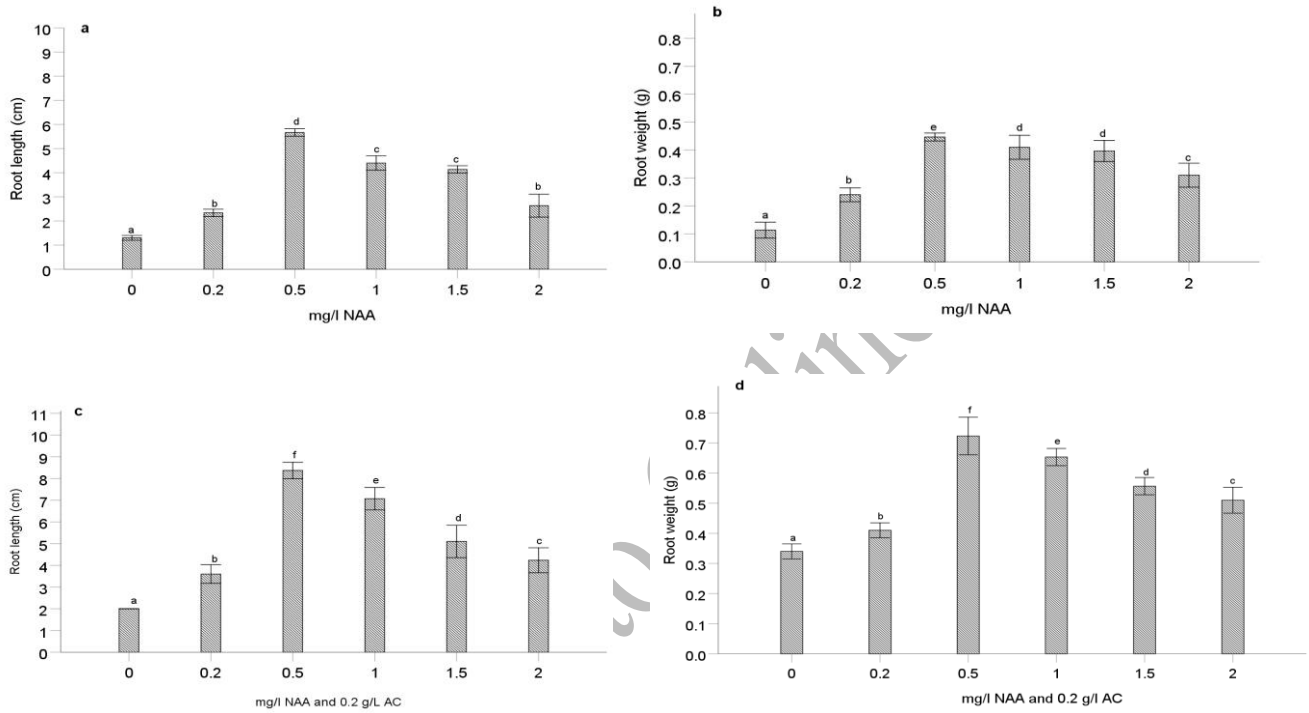

Fig. 4 Effects of NAA and Activated Charcoal on *in vitro* rooting of *L. salicaria*. a- The roots length on MS media with NAA; b- The weight of roots on MS media with NAA; c- The root length on MS media with NAA and 0.2 g/l Activated Charcoal; d- The weight of roots on MS media with NAA and 0.2 g/l Activated Charcoal

In summary, the study demonstrated that the inclusion of 0.5 mg/L NAA and 0.2 g/L activated charcoal in the culture medium promoted robust rooting in *L. salicaria* micro shoots, resulting in a higher percentage of rooted shoots and the development of thick roots. Moreover, this treatment yielded longer roots and greater root biomass compared to the control group. These findings highlight the significance of NAA and activated charcoal in enhancing the root establishment and growth of *L. salicaria*, offering valuable insights for propagation and cultivation practices.

Following the successful rooting of *L. salicaria* microshoots, the next crucial step in the study was to acclimatize the plantlets and ensure their survival in a soil composition suitable for their continued growth. The results revealed that an impressive 100% of the plantlets were able to adapt and thrive after being transferred to plastic pots containing a specific soil composition.

The soil composition used consisted of a mixture of cocopeat, perlite, and vermicomposting in ratios of 50%, 25%, and 25%, respectively. This combination was carefully selected to provide the necessary nutrients, moisture

retention, and aeration for optimal plant growth. The successful acclimatization of all plantlets indicates that this soil composition effectively supports the establishment and development of *L. salicaria*.

Upon transfer to the plastic pots, the plantlets displayed rapid shoot length growth, as illustrated in Fig. 3f. This visual representation further highlights the adaptability and vigor of the plantlets in their new environment. The observed rapid shoot length growth serves as a positive indicator of the health and vitality of the acclimatized *L. salicaria* plantlets.

The two-week survival period following transfer to the plastic pots is noteworthy, as it demonstrates the resilience and ability of *L. salicaria* to establish itself in a new growth medium. This successful acclimatization is an essential milestone in the cultivation process, as it paves the way for further growth, development, and potential applications of *L. salicaria* in various agricultural, horticultural, or ecological contexts.

DISCUSSION

In this experiment, the effects of activated charcoal (AC) on the propagation of *L. salicaria* were investigated. Various concentrations of activated charcoal were employed, and the results showed significant improvements in both shoot length and the number of shoots compared to the control group. These findings demonstrate the positive impact of activated charcoal on shoot proliferation in *L. salicaria*, as illustrated in Table 1. Activated carbon has a high adsorption capacity, allowing it to remove inhibitory compounds present in the culture medium [18]. In *in vitro* cultures, certain phenolic compounds and allelochemicals released by plant tissues or microbial contaminants can inhibit shoot growth [19]. Activated carbon adsorbs these inhibitory compounds, preventing their negative effects and promoting shoot development [20]. Moreover, activated carbon serves additional functions, including the reduction of oxidative stress, preservation of nutrients, enhancement of plant hormone activity, and facilitation of microbial interactions [21, 22]. The beneficial effects of activated charcoal on plant growth have been consistently reported in other studies as well [23, 24]. These studies have consistently demonstrated that activated charcoal plays a beneficial role in enhancing shoot proliferation and development across various plant species. For instance, Buckseth *et al*. [25] conducted a study examining the effects of activated charcoal on the *in vitro* growth and development of *Solanum tuberosum* L. They observed significant results for shoot length, with concentrations of 100 and 200 mg/L of activated charcoal leading to a considerable increase in shoot length of the Kufri Jyoti cultivar. Similarly, benmahioul *et al.* [26] observed a positive effect of activated charcoal on the length of the aerial part of *Pistacia vera*. These findings provide further support to the notion that activated charcoal promotes shoot growth, as observed in the current study with *L. salicaria*.

Specifically, in this study, the culture medium containing 0.5 g/L of activated charcoal yielded the highest shoot length (7.1 cm ± 0.15) and several shoots (2.4 ± 0.11) among the different concentrations tested (Table 1). However, it should be noted that excessively high concentrations of activated charcoal (above 0.5 g/L) were found to have a detrimental effect on shoot length and number. This negative impact can be attributed to potential nutrient reduction in the culture medium. high concentrations or prolonged exposure to activated carbon can exert toxic effects on plant tissues. These toxic effects can manifest as cellular damage, reduced metabolic activity, or impaired physiological processes, ultimately inhibiting shoot growth [20, 22]. Komalavalli and Rao [27] conducted a study on *Gymnema sylvestre* and observed a decrease in the number of shoots when cultured in a medium containing activated charcoal. Similarly, activated charcoal was found to have a negative effect on the microspore culture of *Brassica juncea* [28]. These findings emphasize the significance of carefully determining the appropriate concentration of activated charcoal for each specific plant species, as excessively high concentrations may have adverse effects on shoot growth. Therefore, it is crucial to strike a balance and optimize the concentration of activated charcoal for maximum shoot proliferation and development, considering the specific requirements of the plant species under investigation. By doing so, the full potential of activated charcoal can be harnessed for successful plant propagation.

In this research, the effect of activated charcoal (AC) on the number of leaves in *L. salicaria* was investigated. The results showed that the maximum number of leaves (15.2±0.29) was obtained when the culture medium was supplemented with MS+0.75 g/L AC, as compared to the control group (Table 1). This finding suggests a positive effect of AC on leaf growth in *L. salicaria.* Activated carbon helps conserve nutrients in the culture medium. It can adsorb excess nutrients and release them when needed, ensuring a balanced nutrient supply for leaf growth.

This nutrient conservation mechanism promotes healthy leaf development and enhances overall plant vigor [23]. Further supporting this notion, Poniewozik *et al*. [15] conducted a study on *Paphiopedilum insigne* and reported that the addition of 1 g/L AC had a significant impact on the development of larger leaves in the plant. Interestingly, they observed a reduction in the number of o-dihydroxyphenols in the leaves of *Paphiopedilum insigne*, suggesting that activated charcoal can alter the chemical composition of the leaves, leading to enhanced leaf growth. However, it is important to note that at higher concentrations of AC (≥1 g/L), a decrease in the number of leaves was observed (Table 1). The reduction in leaf count at higher AC concentrations can be influenced by various factors [29]. Nolan *et al*. [30] propose that the chemicals released by activated charcoal may have a direct effect on the plant's response, leading to a decrease in leaf production. Additionally, AC can influence plant growth parameters, including the number of leaves, through the non-selective absorption of certain substances from the culture medium [22]. The interaction between AC and the plant's physiological processes may vary depending on the concentration and duration of exposure, leading to differential effects on leaf development. Therefore, it is crucial to carefully determine the appropriate concentration of activated charcoal to optimize leaf growth in *L. salicaria*. By maintaining an optimal concentration, the positive effects of AC on leaf development can be maximized while avoiding any negative impacts associated with excessively high concentrations. This research provides valuable insights into the role of activated charcoal in leaf growth and highlights the need for further investigation to fully understand the mechanisms underlying its effects on plant physiology and development.

The findings of the present study indicate that the inclusion of activated charcoal (AC) in combination with NAA (naphthaleneacetic acid) significantly influenced root development in *L. salicaria*. The weight and length of roots were notably increased in the culture medium supplemented with NAA and AC (Fig. 4). Among the different treatment combinations, it was observed that the optimal MS culture medium for root development in *L. salicaria* was 0.5 mg/L NAA combined with 0.2 g/L AC (Fig. 4, c, d). This particular combination demonstrated superior efficacy in promoting robust root growth in *L. salicaria* compared to other treatment groups. The research conducted by Chen *et al*. [31] supports the notion that activated charcoal positively impacts root development. They investigated the effects of AC on Paeonia suffruticosa and proposed that the enhancement of root growth in media containing AC could be attributed to the stimulation of gene expression associated with the phenylpropanoid biosynthesis pathway. It implies that activated charcoal may exert its influence on root development by influencing specific genetic pathways involved in root growth and development. In another study by Nguyen *et al*. [23], which focused on the *in vitro* regeneration of *Paphiopedilum vietnamense*, it was found that 1/2 MS medium supplemented with 0.5 mg/L NAA and 1 g/L AC resulted in the highest rooting percentage of 88.89%. This further confirms the positive impact of combining NAA and AC on rooting efficiency in different plant species, as observed in both *L. salicaria* and *Paphiopedilum vietnamense.*

Interestingly, the present study also revealed that higher concentrations of NAA (1, 1.5, 2 mg/L) in the culture medium led to a reduction in root length and weight in *L. salicaria* (Fig. 4). This suggests that while NAA is crucial for initiating root growth, excessive concentrations can have inhibitory effects on overall root development. Hence, it is important to carefully determine the appropriate concentration of NAA to optimize root growth without impeding overall root production.

Based on the results obtained, the recommended culture medium for *in vitro* rooting of regenerated *L. salicaria* shoots was determined to be 0.5 mg/L NAA + 0.2 g/L activated charcoal. This specific medium was found to promote the development of long and branched roots, which are highly suitable for successful transplantation to potting soil (Fig. 3c).

These findings provide valuable insights for improving the micropropagation techniques and optimizing the rooting process in *L. salicaria*. By understanding the beneficial effects of activated charcoal in combination with NAA on root development, researchers and horticulturists can apply these findings to enhance the quality and efficiency of plant propagation methods.

The acclimatization process of the tissue-cultured plants to potting soil was successful, with a 100% survival rate and morphologically uniform plants comparable to the control group (Fig. 4f). These results confirm the viability and effectiveness of the propagation method, demonstrating its potential for mass plant production. The

acclimatized plants' ability to thrive in natural conditions signifies their adaptability and suitability for commercial use in various agricultural and horticultural applications.

This study represents a significant milestone as it provides the first comprehensive report on the effects of activated charcoal on the *in vitro* micropropagation of *L. salicaria*. By demonstrating the positive impact of activated charcoal on both shoot and root growth in *L. salicaria*, this study highlights its potential as a valuable tool for optimizing micropropagation techniques. Understanding the specific effects of activated charcoal on shoot proliferation, shoot length, number of shoots, root development, and root weight in *L. salicaria* provides a foundation for future research and practical applications. These insights can inform the development of more efficient and effective micropropagation protocols for *L. salicaria* and potentially other plant species as well.

CONCLUSION

This study represents a protocol in the field of *in vitro* propagation of *L. salicaria* by establishing an efficient and cost-effective protocol. The utilization of activated carbon proved to be pivotal in promoting the growth and multiplication of shoots in this plant species, as confirmed by the results obtained.

The inclusion of activated carbon in the culture medium was found to have a profound positive impact on shoot growth and multiplication in *L. salicaria*. This innovative approach not only enhanced the overall propagation process but also facilitated the development of a greater number of healthy shoots, thereby increasing the yield of desired plant material.

Furthermore, the combination of NAA and activated carbon, specifically with 0.5 mg/L NAA and 0.2 g/l activated carbon, emerged as a highly effective treatment for stimulating secondary root production and augmenting the length of both primary and secondary roots in *L. salicaria*. This combination of growth regulators and activated carbon demonstrated its ability to significantly enhance root system development, which is essential for the establishment and subsequent growth of plants in various environments.

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Conflicts of Interest

The authors confirm that there are no conflicts of interest.

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