Influence of Relative Humidity on the Survivability of Mulberry Mini Clones Propagated Using Mini Clonal Technology

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ABSTRACT

Mulberry (*Morus spp*.) is a critical plant for sericulture, serving as the exclusive feed for the silkworm (*Bombyx mori* L.). The propagation of high-quality, genetically uniform mulberry plants is essential to meet the growing demand for sericulture. Mini clonal technology has become a reliable method for large-scale propagation of mulberry thereby offering efficient multiplication of elite clones. However, the success of mini clonal technology is highly dependent on environmental factors particularly relative humidity (RH). RH plays a vital role in various physiological processes including water retention, transpiration and root development which will directly influence the survival of mulberry mini clones during propagation. This article explores the complex interactions between RH and plant physiology and their collective impact on the survivability of mulberry mini clones propagated through mini clonal technology.

INTRODUCTION

Mulberry (*Morus* spp.) is integral to the sericulture industry, providing the leaves essential for the silkworms that produce silk. The global demand for silk has led to the need for increased production of high-quality mulberry plants thus prompting the development of innovative propagation techniques. Mini clonal technology has emerged as a key method for the rapid and large-scale propagation of mulberry plants. This technology involves the use of mini cuttings, often treated with rooting hormones to produce genetically identical plants under controlled conditions (Parthiban *et al.*, 2017).

The success of mini clonal propagation is influenced by several environmental factors such as relative humidity (RH) being one of the most critical. RH affects transpiration rates, water availability and nutrient absorption, all of which are fundamental to the successful establishment of mini clones. During the rooting and acclimatization phases, maintaining optimal RH is essential for promoting root formation, minimizing water stress and enhancing the overall survival of the clones (Bharathi *et al.*, 2024). This article focus on the importance of RH in the survivability of mulberry mini clones thus examining its physiological effects and the best practices for managing RH in controlled propagation environments.

Mini Clonal Technology

Mini clonal technology is a form of vegetative propagation that utilizes mini cuttings from a mother plant to create new, genetically identical plants. It is widely used in the propagation of mulberry due to its ability to produce uniform plants with desirable traits such as disease resistance, drought tolerance and high leaf yield (Kiruthika *et al.*, 2020). The process typically involves the following steps:

- **Selection of Mother Plants**: Healthy, genetically superior plants are chosen as the source for cuttings.
- **Collection of Cuttings**: Small cuttings which are typically 12-15 cm in length were taken from the mother plants. These cuttings should contain active apical node and a small amount of terminal leaf.
- **Rooting Phase**: The cuttings are placed in a controlled environment where temperature, humidity and light are regulated to promote root development.
- **Acclimatization and Hardening**: After rooting, the mini clones undergo acclimatization (hardening), where they are gradually exposed to outdoor conditions before transplantation.

The entire process is sensitive to environmental variables particularly RH, which plays a vital role in the moisture dynamics of the cutting during the rooting and hardening stages.

Relative Humidity and Its Role in Plant Physiology

Relative humidity (RH) is defined as the amount of moisture in the air compared to the maximum amount of moisture the air can hold at a given temperature (Bharathi *et al.*, 2024). RH directly influences several physiological processes in plants including:

- **Transpiration**: The process by which water is absorbed by the roots transported through the plant and released into the atmosphere through the stomata. RH regulates the rate of transpiration and lower RH levels result in higher transpiration rates.

- **Water Retention**: Higher RH reduces transpiration thus allowing plants to retain more water which is crucial during the early stages of propagation, when the root system is not yet fully developed.
- **Stomatal Regulation**: RH affects the opening and closing of stomata which are responsible for gas exchange. In low-humidity conditions, plants may close their stomata to conserve water thereby potentially reducing photosynthesis and growth.

Understanding how RH interacts with these processes is crucial for optimizing the survivability of mulberry mini clones during propagation (Parthiban *et al.*, 2017).

Influence of Relative Humidity on Mulberry Mini Clones

1. Water Balance and Transpiration

During the initial stages of propagation, mulberry cuttings are highly vulnerable to water stress. Since they lack a fully developed root system, they rely on their ability to minimize water loss through transpiration. High RH environments (typically 80-95%) are necessary to reduce transpiration rates and maintain water balance within the cuttings. Under low RH conditions, water loss through transpiration can exceed the water absorbed by the cutting thus leading to wilting, desiccation and reduced survival rates (Sulichantini *et al.*, 2024).

Mulberry mini clones propagated in environments with optimal RH experience reduced water stress thus allowing them to maintain turgidity which is essential for cell expansion and growth. High RH also supports the development of the root system by creating a favourable moisture gradient that encourages water uptake through the stem and leaves (Sharma *et al.*, 2012).

2. Root Development

The rooting phase is one of the most critical stages in the propagation of mulberry mini clones. During this phase, the cutting transitions from relying on external water supply (from misting or fogging systems) to developing its own root system for water and nutrient absorption. High RH facilitates this process by preventing excessive water loss and ensuring that the cutting remains hydrated. It also promotes cell division and elongation in the root initiation zone thus leading to faster and more robust root development (Bharathi *et al.*, 2024).

In contrast, low RH environments can impede root formation by causing dehydration and cell shrinkage. Cuttings exposed to low RH may experience delayed or incomplete root development thereby reducing their chances of survival once transplanted to the field (Kuppusamy *et al.*, 2019).

3. Nutrient Uptake and Photosynthesis

High RH levels support better nutrient uptake during the propagation process. Since the cutting lacks an established root system during the early stages of mini clonal propagation, nutrient absorption occurs primarily through the leaves and stems. High humidity ensures that the cells remain hydrated thus allowing for more efficient absorption of nutrients from the surrounding environment. Additionally, optimal RH levels promote stomatal opening which is crucial for photosynthesis. This process provides the energy needed for growth and root development (Bharathi *et al.*, 2024).

Low RH levels, on the other hand will cause the stomata to close thereby reducing photosynthesis and limiting the energy available for root and shoot growth. This can lead to stunted growth and decreased survival rates (Sulichantini *et al.*, 2024).

Optimal Relative Humidity Levels for Mulberry Mini Clones

Research and field trials have demonstrated that maintaining optimal RH levels is critical for the successful propagation of mulberry mini clones. The following RH ranges are considered ideal for different stages of the propagation process:

- **Rooting Phase**: During the rooting phase, RH levels should be maintained between 80-95%. High humidity ensures that the cuttings remain hydrated and minimizes water loss through transpiration. Misting or fogging systems are often used to maintain these humidity levels in propagation chambers (Kiruthika *et al.*, 2020).
- **Acclimatization Phase**: As the clones transition from the controlled environment to outdoor conditions, the RH levels should be gradually reduced to around 60-70%. This gradual reduction helps the clones adjust to lower humidity levels without experiencing water stress or shock. The hardening process should take place over a period of 7-15 days depending on the environmental conditions and vary among crops (Kuppusamy *et al.*, 2019).
- **Post-Transplantation Phase**: Once the clones are transplanted to the field, they should be exposed to natural RH conditions. However, ensuring adequate irrigation and mulching can help maintain soil moisture levels and support the clones during this critical phase (Bharathi *et al.*, 2024).

Maintaining these optimal RH levels ensures that mulberry mini clones can successfully develop roots, acclimatize to outdoor conditions and survive transplantation to the field (Bharathi *et al.*, 2024).

Effects of Suboptimal Relative Humidity on Clone Survivability

Suboptimal RH levels, whether too high or too low can significantly impact the survivability of mulberry mini clones. Understanding these effects is crucial for optimizing the propagation process and minimizing losses.

1. Low Relative Humidity

Low RH levels (<60%) can lead to excessive transpiration thereby causing the cuttings to lose more water than they can absorb. This water loss leads to wilting, dehydration and in severe cases, death. The following are some specific effects of low RH on mulberry mini clones:

- **Dehydration and Wilting**: Low RH causes rapid water loss through transpiration thereby leading to a reduction in turgor pressure. This results in wilting and a loss of structural integrity in the leaves and stems.
- **Delayed Rooting**: Dehydrated cuttings are less likely to develop roots as the cells responsible for root initiation become stressed and lose their ability to divide and elongate.
- **Reduced Photosynthesis**: Low humidity levels cause stomata to close thus limiting CO_2 uptake and reducing the rate of photosynthesis. This results in lower energy availability for root development and growth (Bharathi *et al.*, 2024).

2. High Relative Humidity

While high RH levels are generally beneficial during the rooting phase, excessively high humidity (>95%) can also pose risks. Prolonged exposure to extremely high humidity can create conditions favourable for the growth of pathogens particularly fungal and bacterial diseases (Kuppusamy *et al.*, 2019). Specific effects of excessive RH include:

- **Increased Risk of Fungal Diseases**: High humidity levels create a moist environment that encourages the growth of fungal pathogens such as *Botrytis* sp. and *Rhizoctonia* sp. These diseases can cause damping-off, stem rot and root rot, all of which reduce the survival rates of the clones.
- **Reduced Oxygen Availability**: Extremely high humidity can reduce gas exchange thus leading to lower oxygen availability around the cuttings. This can slow down root respiration and reduce root formation.
- **Delayed Acclimatization**: If the RH is not gradually reduced during the hardening phase, the clones may struggle to adjust to lower humidity levels once transplanted to the field. This can lead to shock and reduced survival rates (Sharma *et al.*, 2012).

Best Practices for Managing Relative Humidity in Mini Clonal Propagation

To maximize the survivability of mulberry mini clones, it is essential to manage RH levels effectively throughout the propagation process. The following best practices can help optimize humidity management:

1. Misting and Fogging Systems

Misting and fogging systems are commonly used in propagation chambers to maintain high humidity levels. These systems deliver fine droplets of water to the air thus creating a humid environment around the cuttings without causing waterlogging. Automated systems that adjust misting frequency based on ambient RH levels can ensure that the cuttings receive consistent moisture (Sulichantini *et al.*, 2024).

2. Humidity Monitoring

Accurate monitoring of RH levels is critical for maintaining the ideal environment for mulberry mini clones. Hygrometers and humidity sensors should be placed throughout the propagation chamber to monitor RH levels in real-time. If RH levels drop too low, misting or fogging systems can be activated to restore optimal conditions (Bharathi *et al.*, 2024).

3. Gradual Acclimatization

As the clones develop roots, it is important to gradually reduce RH levels during the hardening phase. This can be achieved by adjusting the misting system to reduce the frequency of misting and allowing the clones to experience lower humidity levels. This gradual reduction helps the clones build tolerance to outdoor conditions and improves their survival rates after transplantation (Kuppusamy *et al.*, 2019).

4. Pathogen Control

To prevent fungal and bacterial diseases in high-humidity environments, it is important to maintain good air circulation in the propagation chamber. Fans can be used to ensure that the air does not become stagnant which helps reduce the risk of pathogen growth. Additionally, disinfecting the propagation chamber and using disease-resistant varieties of mulberry can help minimize the impact of pathogens (Sharma *et al.*, 2012).

CONCLUSION

Relative humidity is a critical factor influencing the survivability of mulberry mini clones propagated using mini clonal technology. High RH levels during the rooting phase promote water retention, reduce transpiration and support root development while lower RH levels during the acclimatization phase help the clones adjust to outdoor conditions. Maintaining optimal RH levels through effective humidity management is essential for maximizing the survival rates of mulberry mini clones. By understanding the physiological effects of RH on plant development and implementing best practices for humidity control, growers can improve the success of mulberry propagation and ensure a reliable supply of high-quality plants for sericulture and other industries dependent on mulberry cultivation.

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