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Understanding and Controlling Agrobacterium tumefaciens Overgrowth in Plant Transformation: Causes and Solutions

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Abstract:

Various challenges significantly impact the transformation process, both directly and indirectly. One major concern is the overgrowth of Agrobacterium, which can occur after the co-cultivation phase of the explant. This issue has been observed in several plant species and can disrupt the entire transformation process. To address this problem, multiple strategies are being employed to prevent the unwanted bacterial growth. Once overgrowth occurs, it becomes extremely difficult to rectify, making prevention crucial. Several factors play a role in regulating this phenomenon, including the nature of the explant, the A. tumefaciens strain, T-DNA vector, co-cultivation conditions (time and environment), the use of acetosyringone, washing medium, and antibiotics (type, concentration, combination, and incubation period). In this article, we review these factors based on available research reports.

Keywords: Agrobacterium tumefaciens, Overgrowth, Plant transformation, Genetic modification, Transformation efciency, Co-cultivation.

Introduction:

Agrobacterium tumefaciens-mediated transformation is a widely used method for plant genetic manipulation[1], both for producing genetically modified crops and for functional genomic studies [2]. However, a major challenge in such experiments[3] is achieving high transformation efficiency [4], which depends on various factors such as plant species [5], genotype, type of explant, media pH [6], regeneration and co-cultivation conditions, plant growth regulators, antibiotics, temperature, light [7], A. tumefaciens strain, cell density, gene construct, cell competence after wounding, and control of A [8]. tumefaciens overgrowth. A. tumefaciens overgrowth is a serious problem [9] in the transformation process and can lead to unsuccessful transformations or adversely affect the host plant [10]. It is crucial to understand and address this issue through a step-by-step analysis of the entire transformation process [11].

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A graphic representation of standard plant transformation steps is provided, starting from A [12]. tumefaciens inoculation on the explant [13] to be transformed, followed by the co-cultivation period [14]. During this process, the host material faces significant stress [15], resulting in an excess population [16] of the bacterium known as A [17]. tumefaciens overgrowth. This overgrowth can persist [18] from co-cultivation to the regeneration stage and requires continuous use of high-dose[19] antibiotics for elimination. To ensure successful[20] transformation and prevent the escape of genetically modified A [21]. tumefaciens into the environment, it is essential to establish a consistent protocol for eradicating viable A. tumefaciens cells[22]. In this article, we discuss A. tumefaciens overgrowth as a consequence of various factors involved in the transformation process, rather than a mere cause of lower transformation efficiency [23].

The issue of Agrobacterium tumefaciens overgrowth in plant genetic transformation studies has been reported frequently and can significantly reduce the efficiency of the transformation process. This problem is not limited to specific plant species [24], A. tumefaciens strains, or types of transformation. Several factors contribute to A. tumefaciens overgrowth, and these factors need to be regulated to prevent this unwanted growth [25].

Explant nature:

The type, size, maturity, regeneration ability, genotype, and handling ease of the explant influence its susceptibility to A [26]. tumefaciens overgrowth. Callus explants are more vulnerable, and dense micro wounds on citrus leaves can lead to bacterial overgrowth [27].

A. tumefaciens strain and T-DNA vector:

Different A. tumefaciens strains have varying virulence and overgrowth potential. Strains like EHA 101 and AGL1 are more prone to overgrowth and may require higher antibiotic doses for suppression [28].

Co-cultivation conditions:

The co-cultivation period is crucial for A. tumefaciens to deliver DNA into the host genome. However, this period can also lead to overgrowth [29]. Proper optimization of co-cultivation time and conditions is necessary to avoid overgrowth [30].

Antibiotics:

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Antibiotics are used to eliminate A [31]. tumefaciens after co-cultivation. However, using the correct type, concentration, and combination of antibiotics is crucial to ensure effective suppression of overgrowth without harming the host plant [32].

Washing medium:

Proper washing of co-cultivated explants with antibiotic solutions can help remove excess A. tumefaciens cells and prevent overgrowth [33].

Temperature and light:

Environmental conditions during the transformation process can influence bacterial growth [34]. Proper control of temperature and light is important to avoid promoting overgrowth[35].

Surface area of infection:

The density of trichomes or micro wounds on the explant's surface can affect bacterial growth [36]. Special attention is needed when dealing with explants with a higher surface area of infection[37].

Genotype variability:

Different genotypes may show variations in infection susceptibility, and choosing the right genotype can play a role in avoiding overgrowth [38].

Overall, an efficient transformation protocol relies on carefully considering and regulating these factors to prevent A [39]. tumefaciens overgrowth during the transformation process. Proper optimization and control of each parameter can help achieve successful plant transformation with high efficiency [40].

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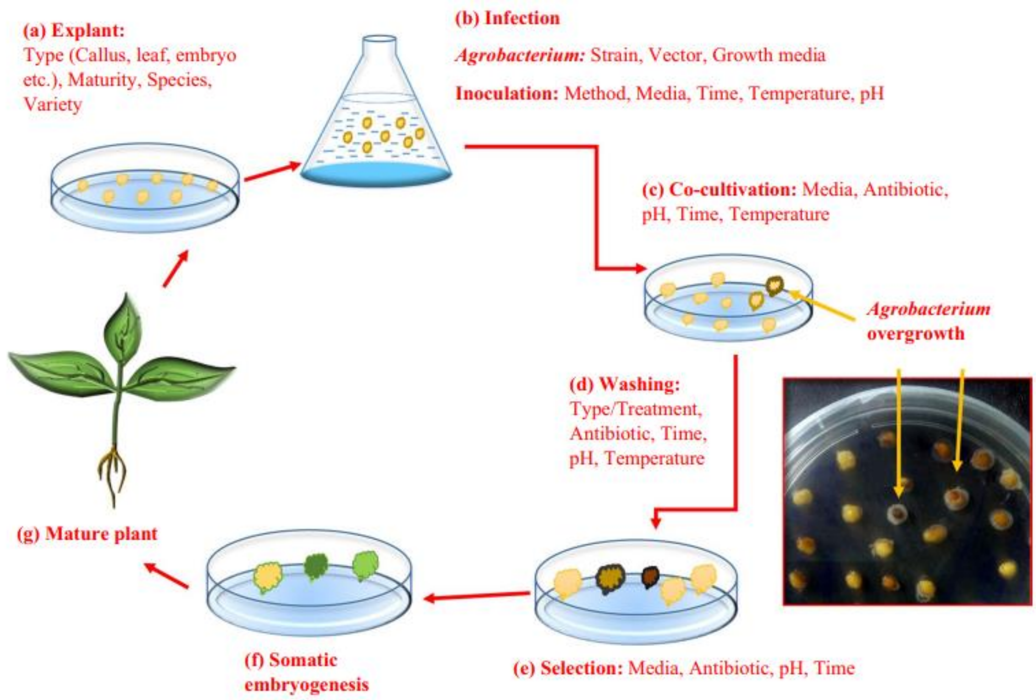

Fig. 1 Factors affecting *Agrobacterium* overgrowth in explant during plant transformation

Conclusion:

In conclusion, A. tumefaciens overgrowth remains a significant challenge in plant transformation, affecting the efficiency of the process across different plant species and genotypes. Preventative measures are more reliable than cures, and careful attention should be given to various factors during the transformation process to minimize the risk of overgrowth. Explant nature, A. tumefaciens strain, T-DNA vector, co-cultivation conditions, antibiotics, and other environmental factors all play crucial roles in determining the likelihood of overgrowth. Efforts should be made to develop new antibiotics that specifically target A. tumefaciens or explore mutant strains that are easier to eliminate. In-depth research is required to better understand the factors responsible for overgrowth and to develop more effective strategies to prevent and control it. The authors acknowledge the financial support provided by the DBT-JRF fellowship to Monoj Sutradhar for the completion of this study. Apologies are extended to researchers whose works could not be cited here due to space and topic constraints.

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