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***Agrobacterium* mediated transformation in *Rubia cordifolia* for obtaining hairy root producing Anthraquinone dye**

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Case Report

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ABSTRACT

Rubia cordifolia is known to contain substantial amount of secondary metabolites such as anthraquinones and dye, especially in the roots. This plant was infected with *Agrobacterium rhizogenes* to transfer the genes *rolA*, *rolB*, *rolC* into the plant genome. It causes tumour formation and hairy root disease in the plant. Thus, it was found that the production of anthraquinones was increased after transfection. In further studies, anthraquinones can be isolated from these roots. There are many applications of the secondary metabolites produced by plants.

INTRODUCTION

The herb *Rubia cordifolia* is usually categorized as GRAS (generally recognized as safe) [1]. *R. Cordifolia* Linn. (Rubiaceae) is commonly known as Indian Madder Manjistha [2]. It is a perennial, herbaceous climber. The roots are 4-8 cm long, reddish, cylindrical, flexuous, with a thin red bark. Stems often have a long, rough, grooved, woody base. The family Rubiaceae comprises about 450 genera and 6500 species and includes trees, shrubs and infrequently herbs.

Plants belonging to this family are known to contain substantial amounts of anthraquinones, especially in the roots. It purifies the blood by removing toxins from the blood, dissolving obstructions in blood flow and removing stagnant blood. The *rolA*, *rolB* and *rolC* genes are plant oncogenes that are carried in plasmids of the plant pathogen *Agrobacterium rhizogenes*. Following agrobacterial infection, these genes are transferred into the plant genome and cause tumor formation and hairy root disease. A new function for the *rol* genes in plant- *Agrobacterium* interactions became apparent with the discovery that these genes are also potential activators of secondary metabolism in transformed cells in different plant families [3].

Thus, it becomes apparent that the *rol* genes generate effects which resemble defense reactions of plants. Little is known about mechanisms by which the *rol* genes perturb secondary metabolism. By studying the transgenic for the *rolB* and *rolC* genes callus cultures of *R. cordifolia*, we can find out if anthraquinone production increases in transformed cultures compared with the non-transformed culture [4].

MATERIALS AND METHODS

Media preparation

Already customized Murashige and Skoog (MS) media was prepared with 3% sucrose. The media was adjusted to pH 5.8 prior to autoclaving at 121 °C under 15 lb inch² for 15 min.

Callus inoculation

Callus was procured from the Tissue culture laboratory, VIT University, Vellore. The callus was excised into bits of 0.2–0.5

cm length. The obtained callus was further inoculated on woody plant (WP) media.

Co-cultivation

The callus obtained was subjected to mechanical wound. The wounded callus was suspended in the suspension media supplied with 3% sucrose, for the infection to take place. Later the *Agrobacterium* culture was added to this media for efficient transformation. The infected callus culture was maintained in culture room until the hairy root is obtained.

RESULTS AND DISCUSSION

The callus started to grow after 20 days. The initial hairy root was measured and parameters were analyzed.

The length of the control root and a previously transformed hairy root was compared and it showed that the length of transformed root was 4.7cm as compared to 4.2 cm of the control. Subsequently, the weight of the transformed root (187 mg) was found higher than the control (120 mg) (Figure 1).



Figure 1: Initial callus formation.

Compared to the control, the transformed plant produced minimal proximate levels on anthraquinones (secondary metabolites) in the callus stage itself, while control produced metabolites after growth. The absorbance values were mostly shown at peaks of 602 nm, 410 nm and 350 nm which signified the higher absorbance in the transformed hairy root (Figure 2).



Figure 2: Replicates of callus culture.

The use of hormone free media was found to be enhancing the secondary metabolite production in transformed plant much higher than the normal growth plant.

CONCLUSION

Agrobacterium rhizogenes causes hairy root diseases in plants, which are characterized by high growth rate, genetic stability and growth in hormone free media. They have shown promising higher productivity of valuable secondary metabolites like anthraquinone. The comparative study of the different parameters between the control and transformed plant has shown the significance of hairy root transformation and its importance in the field of dye research.

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