Overcoming phytoplasma infection in Paulownia tomentosa by meristem in vitro culture

Rumyana P. ***Valkova*** [1, *], *Gergana G.* ***Zahmanova*** [1, 2], *Elena D.* ***Apostolova-Kuzova*** [1], *Milena L.* ***Kostova*** [3], *Valentina T.* ***Toneva*** [1,4]

1 University of Plovdiv, Faculty of Biology, Department of Plant Physiology and Molecular Biology; 2 Center of Plant System Biology and Biotechnology, Department of Technology Transfer and IP Management; 3 Agricultural University, Faculty of Agronomy,Department of Plant physiology, biochemistry and genetics; 4 Institute of Molecular Biology and Biotechnologies.

Abstract Paulownia witches' broom (PaWB) is caused by phytoplasma of Aster Yellows group, and resulted in severe damage to the growth and premature death of the infected tree. In vitro cultures of meristems are important tools for solving problems with plant diseases. In recent study meristem cultures from phytoplasma infected *Paulownia tomentosa* have been created in an attempt to obtain healthy plants. The faster procedure with longer explants generated 95% regeneration but no healthy plants. The combination of smaller explants and thermotherapy resulted in the generation of plantlets with a healthy phenotype. The PCR analyzes showed the absence of phytoplasma only in one of the produced plants. Despite the low efficiency, expressed in one phytoplasma-free regenerant of a total of 480 explants, these results can serve to continue the work on optimizing the used strategy.

The aim of the present study was to regenerate healthy plants from PaWB-infected *Paulownia tomentosa* using the meristem cultures technique.

- The first pattern – meristem tips 1-2.5 mm in length;
- The second variant - meristem tips 0.5 mm in length;
- The third variant - explants 0.5 mm in length combined with thermotherapy (donor plants were preliminarily cultivated for one week at 40 ° C).
- The fourth pattern - Combination of callus and meristem culture with explants 1-2.5 mm in length.

Table 1. Regeneration efficiency of meristem cultures

	Number of explants per experiment	Average number of plantlets/callus*	Regeneration efficiency	Total number of produced plantlets with healthy phenotype
Meristem tissue culture from explants 1-2,5 mm in length	40	38±2	95%	0
Meristem tissue culture from explants 0,5 mm in length	40	18±4	45%	0
Thermotherapy and meristem tips - 0,5mm in length	40	14,7±2,309	36,7%	3
*) Meristem tips 1-2,5 mm in length + callus	40	33,7±2,081	84,17%	43

Figure 1. Changes of morphology in *Paulownia tomentosa* plantlets: (A) plant with PaWB infection; (B) plantlets after phytoplasma eradication; (C) plantlets after indirect regeneration from callus tissue with bigger leaves and enlarged internodes like healthy phenotype, but after prolonged cultivation witches' broom symptom appeared (proliferation of axillary shoots).

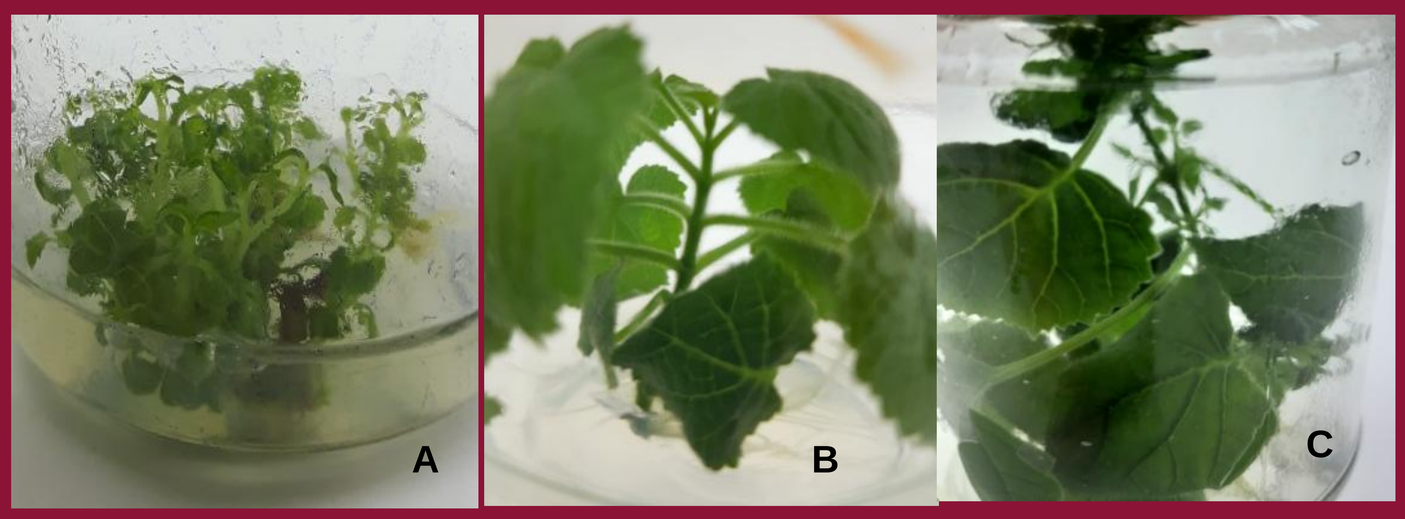
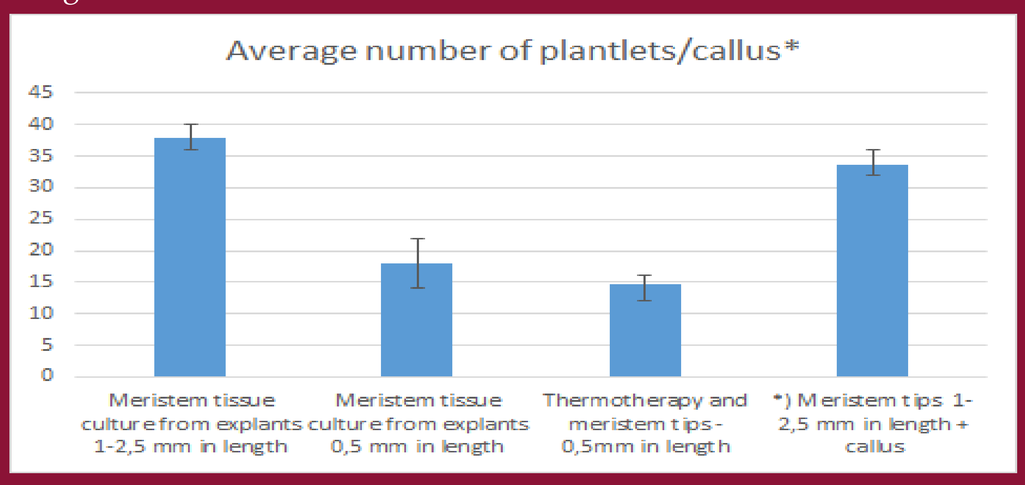
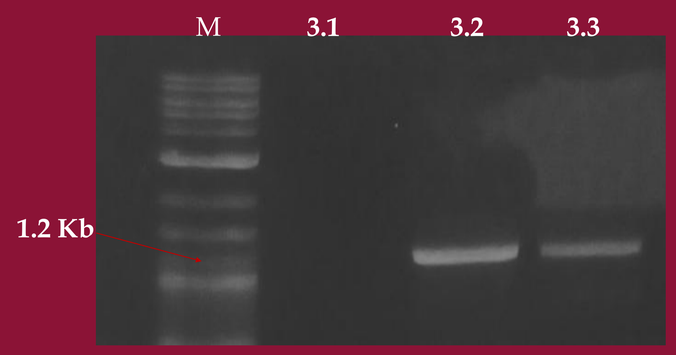


Figure 2. Average number of plantlets/callus* in provided experimental designs



The PCR analysis with universal R16F2/R2 primer pair, from the first newly formed plant with a healthy phenotype, produced through thermotherapy and 0.5 mm meristem-tips, was not amplified phytoplasma' 16S rDNA, but 1.2 kb long products were amplified from the other two asymptomatic samples (fig. 3). The results showed that from 120 explants were produced tree promising samples, but only one was free from phytoplasma, with 0.83% efficiency in overcoming witches' broom infection.

Figure 3. Detection of phytoplasma 16SrDNA by PCR. **M:** DNA Marker; (3.1) the first regenerant with healthy phenotype; **2:** (3.2) the second regenerant with healthy phenotype; (3.3) the third regenerant with healthy phenotype.



Conclusion After conducting four different strategies for meristem cultures, it has been observed the disappearance of PaWB symptoms in 46 produced regenerants of a total of 480 used explants. Mostly, the combination of meristem tissue with indirect regeneration through callus tissue reduced phytoplasma' symptoms (35.8%), compare to variant with thermotherapy (2.5%), and lack of changes in other two patterns. The healing effect turned to be permanent, for one of changed plantlets, and it was a product of combination between smaller explants and thermotherapy. Despite the low efficiency of the tested strategy, the provided data can serve to continue the study for the eradication of phytoplasma infection in paulownia plants. It has been suggested that the indirectly regenerated plants with changed morphology could be appropriate donor plats for further experiments with meristem tips for pathogen eradication.