Examining viroid concentration reduction from suspension cultures: Thermal vs. Chemical treatments

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Abstract – Viroids are considered as the smallest known infectious agents, consisting solely of 240-400 bp long RNA. Under certain conditions they spread quickly and may induce severe physiological and morphological pathologies in the infected plants. Since there is no efficient treatment and absolutely reliable prevention, these tiny creatures can cause a very serious economic damage. Investigating the effects of various treatment methods on the PSTVd viroid concentration and activity in callus suspension cultures of infected *Solanum jasminoides* is one of our main goals within the scope of the VirEx-project (031A400C,supported in the framework of the "KNU-innovativ"- program, BMBF).
Viroids consist of a sophisticatedly folded single-stranded RNA and therefore can mix with and mimic various RNAs of the host cells, which makes their specific elimination from infected cells challenging. We try to address some biophysical peculiarities of the viroid reproduction cycle, requiring intact 3d-structure and certain network of stabilizing hydrogen bonds. The applied treatment methods include low- and high temperature treatment as well as the use of chaotropic agents. An essential part of our work was devoted to the evaluation of treatment efficacy by means of RT-PCR.
Previously, suspension cultures were monitored only till 7- and 11-weeks with temperature- and urea- treatment respectively. A common reason for a monitoring stoppage is an outbreak of contamination, a problem which is almost unavoidable for a long-term cultivation schemes. Our primary aim was to improve quality management and laboratory procedures in order to extend the monitoring up to 12 weeks of treatment and more. Every 2 weeks cells viability, proliferation and sterility were carefully examined. After successful treatment of 8 weeks the results showed a reduction in concentration of viroid. In this experiment, when compared to temperature treatment, urea treated samples were observed more viable.

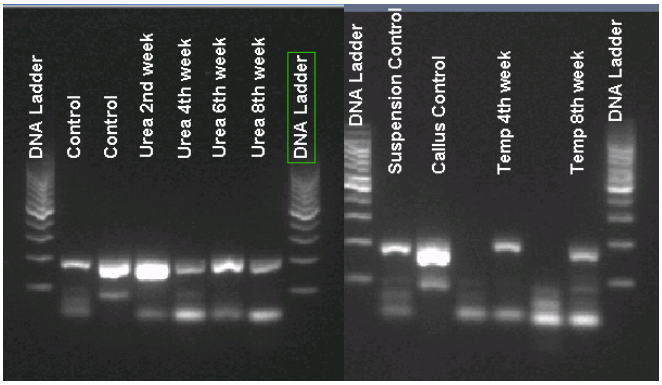

Fig.1.1 Results of 8 weeks urea and temperature treatment.

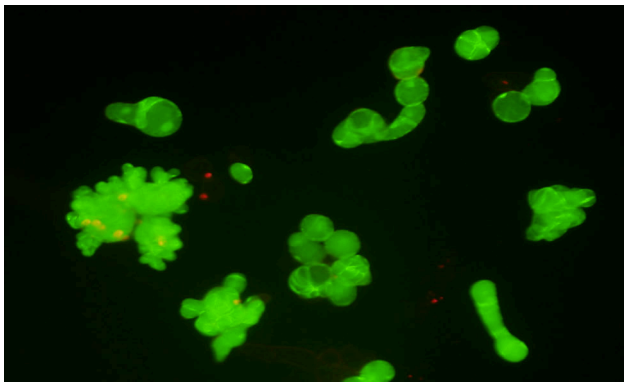

Fig. 1.2 FDA of 4th week of urea treatment

The figures show some achieved results concerning the reduction in PSTVd concentration in the samples after every 2, 4, 6 and 8 weeks of treatment. Fig 1.1 shows the results of 8 weeks urea and temperature treatment. Further treatment of suspension cultures till 12 weeks will be performed. Fig 1.2 shows the suspended *Solanum* cells stained fifth FDA for viability examination of 4th week of urea treated cultures. Green color represents living and healthy cells.

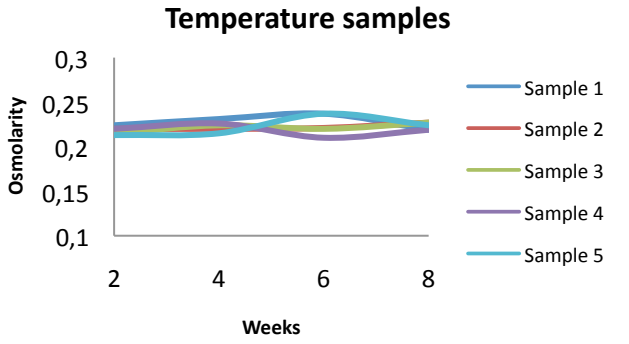

Fig.2. 1

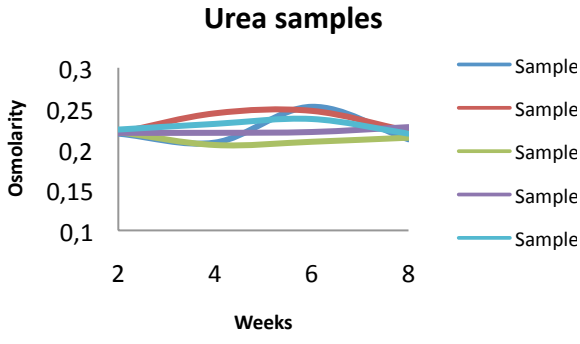

Fig.2. 2

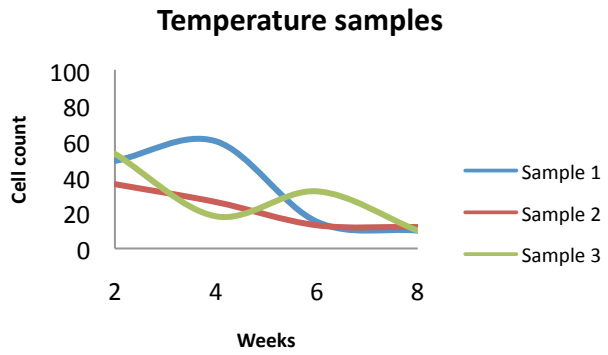

Fig.2. 3

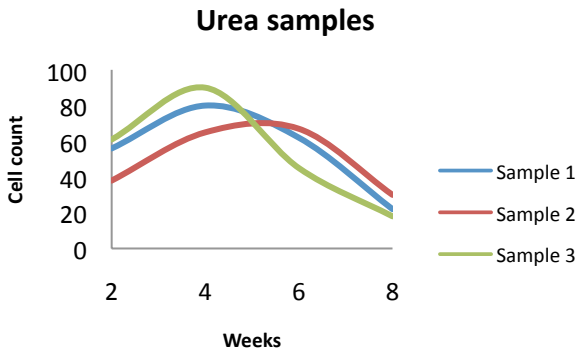

Fig.2. 4

The second block of graphs is indicating the osmolarity and cell count after every 2, 4, 6 and 8 weeks of treatment. Fig 2.1 shows typical dynamics of the suspension medium osmolarity during temperature treatment, Fig2.2 shows such dynamics for the urea treated suspension, Fig2.3 and Fig2.4 shows the consequent cell counts for temperature- and urea treated suspensions, respectively.

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