***In vitro* cultivation, aquaculture and methods of transformation of water caltrop *Trapa* L.**Panfilova M.A.¹, Mikhaylova E.V.², Musin Kh.G.², Kuluev B.R.^{1,2}¹ Bashkir State University, Ufa, Russia; ² Institute of Biochemistry and Genetics UFRS RAS, Ufa, Russia

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Key message. *In vitro* and aquarium cultures of water caltrop were obtained. Optimal conditions of cultivation were determined. The cultures were subjected to *Agrobacterium rhizogenes*-mediated and biolistic transformation.

Keywords: *Trapa natans*, water caltrop, *in vitro*, aquaculture, *Agrobacterium rhizogenes*

Water caltrop *Trapa* L. is an important source of nutrition in warmer regions. In China, India, Japan, Korea the genetics and biologically active compounds of this plant are being actively studied. Its seed coat contains antioxidants and antimicrobial compounds that can be extracted and used in pharmaceutical industry. In Russia and Europe water caltrop is under the risk of extinction. Its only habitats in The Republic of Bashkortostan are lakes Upkankul and Bilgilyar [1,2]. To preserve and study unique local populations, it is important to develop methods of its *in vitro* culture and aquaculture. The goal of our study was to find the perfect conditions for cultivation of water caltrop.

Seeds gathered in September in lake Upkankul were stratified at +4°C for 5 months and then sterilized with ethanol and sodium hypochlorite. In aquarium culture we tried 4 sets of conditions with different pH (8.4 and 6.7) and temperature (22°C and 25°C). 20 L aquariums were filled with tap water. In control conditions natural sapropel was used as a substrate. *In vitro* propagation of water caltrop embryos was carried out on 5 types of MS medium, liquid and solid, rich and poor. 6-BAP, NAA and GA₃ were used to stimulate the development of embryos. Water caltrop *in vitro* cultures are known to suffer from browning. To overcome it, ascorbic and citric acids, AgNO₃ and phloroglucinol were used. The cultures were grown in climate control chamber at 25°C.

In aquarium best results were obtained from settings with higher temperature and more alkaline pH (60% of seeds germinated). Worst rate of seeds germination was observed in more acidic and warm aquarium (seeds did not germinate). On sapropel up to 80% of seeds germinated which is indicative of an important role of organic compound in growth and development of water caltrop. The development of the embryos began after 4-7 days of cultivation. Up to 50% of the embryos germinated depending on the medium composition. More alkaline pH and addition of AgNO₃ and phloroglucinol were the most effective against browning. Some plants were able to flower in these conditions. Transformation of water caltrop *in vitro* and aquarium cultures with *Agrobacterium rhizogenes* strain A4 was unsuccessful. Germinated embryos were also used for biolistic transformation with a plasmid containing *rol* genes isolated from these bacteria.

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