

## The Role of Salicylic Acid in Macrophyte (*Typha angustifolia* L.) Adaptation to Nitrate Nitrogen

Anna A. Ratushnyak, Kseniya I. Abramova and Rifgat R. Shagidullin

State Budgetary Establishment Research Institute for Problems of Ecology  
and Mineral Wealth Use of Tatarstan Academy of Sciences

**Abstract:** Salicylic acid participates in many physiological reactions of plants. This article was intended to study the effects of salicylic acid on growth, biomass production, the potential seed productivity and other characteristics in aquatic macrophyte (*T. angustifolia* L.) in conditions of pollution with nitrate nitrogen. It was detected that an amount of nitrates decreases thereby stimulating a good quality of natural water and increasing diatoms. The obtained results provide a new data on mechanisms of systemic resistance of biological objects. It is possible to say now on practical utilization of salicylic acid in the management of structure of aquatic organisms.

**Key words:** Salicylic acid • Nitrate nitrogen • Macrophyte • Adaptation • Biomass • Phytoplankton

### INTRODUCTION

Salicylic acid participates in many physiological reactions of plants [1]. This chemical may be involved into defense reactions in plants via stimulation of hydrogen dioxide production [2]. Besides that, salicylic acid helps to plants to adapt to salt stress [3], heavy metals [4] and other stressors [5, 6]. However, majority of research works were performed on terrestrial plants. This article was intended to study the effects of salicylic acid on growth, biomass production, the potential seed productivity and other characteristics in aquatic macrophyte (*T. angustifolia* L.) in conditions of pollution with nitrate nitrogen.

### MATERIALS AND METHODS

Investigations were carried out during 4 vegetation seasons of 2005-2008 in mesocosm conditions including natural water (30 L) with concurrent hydrobionts and curtains of *T. angustifolia*. The latter were taken from Sredniy Kaban Lake (Kazan city, Russia) and had surface area of 0.5 m<sup>2</sup>. Two types of biotopes were simulated-overgrown (with the macrophytes) and open one (without the plants). In June, NaNO<sub>3</sub> was introduced to natural water in concentrations of 1 and 10 maximum allowable concentrations (MAC). Also, salicylic acid (100 µM) was added.

Five variants with various combinations of nitrate nitrogen and salicylic acid for each biotope were made: 1. control-natural water without supplements; 2. natural water + 10 MAC of NaNO<sub>3</sub>; 3. natural water + 1 MAC of NaNO<sub>3</sub> + salicylic acid; 4. natural water + 10 MAC of NaNO<sub>3</sub> + salicylic acid; 5. natural water + salicylic acid. Experiments were performed in a seasonal dynamics (from June till September) in triplicates.

Calculations of biomass of overground and underground parts of *T. angustifolia* were by weight method and expressed in grams of dry weight per 1 m<sup>2</sup>. The potential seed productivity was assessed by methodic by Gorbik.

To reveal features of formation of protein, lipid and polysaccharide complexes in plants, radiocarbon method was applied. Radioactive label (water solution of <sup>14</sup>C-acetate) was introduced with syringe into leaf tip. A month later, headstock of wet plant material from overground parts (leaves and stalk) was crumbled with solvents: for isolating pigment complex-with acetone (80%), for isolating proteins-with potassium alkali (0.2 M). Residual of the extract contained polysaccharide complex. Delta-300 counter was used to determine radioactivity in the obtained fractions and an aliquots (5 ml of scintillation liquid and 0.2 µL of extract).

Phytoplanktonic, hydrochemical analysis and statistical treatments were performed according to standard procedures.

## RESULTS AND DISCUSSION

It was found that salicylic acid activated production processes in the macrophyte at the increase level of nitrate nitrogen. A number of stalks per 1 m<sup>2</sup> of plants were increased: 24 vs 14 in control, 1 MAC + salicylic acid-19 vs 17 at 1 MAC, 10 MAC + salicylic acid-24 vs 22 at 10 MAC. Heights of plants (in cm) were also enlarged: 175.0 ± 10.7 vs 140 ± 7.0 in control; 1 MAC + salicylic acid-177 ± 152 vs 150.0 ± 7.5 at 1 MAC; 10 MAC + salicylic acid 185.0 ± 13.1 vs 162.0 ± 8.1 at 10 MAC. Changes in plant biomass are indicated at Figure 1.

Addition of salicylic acid did not influence the potential seed productivity (208.4 ± 21.0 x 10<sup>3</sup> vs 207.4 ± 10.4 x 10<sup>3</sup> in control). At background of 1 MAC and 10 MAC of nitrate nitrogen, we detected 1.5-fold and 1.8-fold decreasing this parameter, respectively.

Salicylic acid stimulated formation of protein fraction in overground parts of the macrophyte-proportion of radioactive label in the substrate was 59.2 ± 4.4 %. This is in agreement of data by Tarchevsky [7]: salicylic acid stimulated formation of proteins in pea seedlings at stress. We detected that label amount in polysaccharides was 30.9 ± 5.0 %, while in lipids-9.9 ± 3.9 %.

We also found that at the community ecology level, salicylic acid changed a structure of phytoplankters. In the open waters (without macrophytes) this effect was not detected (Figure 2).

Addition of nitrate nitrogen in high amounts (10 MAC) to natural water resulted in rearrangements of phytoplankton structure since the first month of investigation in the two types of biotopes.

In the open biotope, blue-green algae (predominantly *Oscillatoria planctonica* Wolosz.) was dominant group in comparison to green algae in control. Their proportion was 98.6-99.5% from total number and 56.5-61.4 % from total biomass (Figure 2).

In overgrown biotope (with macrophytes), we detected changes in algal dominating group. Diatoms were replaced by green algae with active development of *Golenkiniopsis solitaria* Korsch. (92.3 and 73.7%) in comparison to control. In the following months, we detected a restoration of initial structure-diatoms again became a dominating group. In August, a proportion of diatoms were 86.4% (from total number) and 92.8% (from total biomass); in September the corresponding values were 42.3% and 70.9%, respectively.

It was detected that addition of salicylic acid to natural water polluted with nitrate nitrogen (10 MAC) in overgrown biotopes resulted in stimulation and restoration of phytoplankton structure (dominance of diatoms). During a time of investigation, a proportion from total number (in variant 10 MAC + salicylic acid) was 55.9-99.9%. In biotopes without macrophytes, restoration effect was not registered (in variant 10 MAC + salicylic acid blue-green algae were dominating group). This confirms that salicylic acid may activate endo-and exometabolic processes that may alter chemical communication among hydrobionts [7].

Hydrochemical results suggest that addition of salicylic acid to overgrown biotopes resulted in stimulation decreasing concentration of nitrate nitrogen in natural water till lower values: 0.02 mg/L vs 1.7 mg/L (control variant). In other variants, decreasing of nitrate

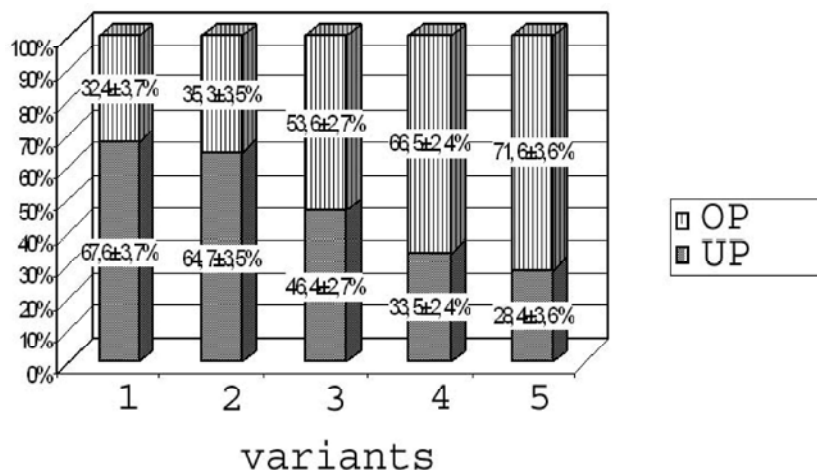


Fig. 1: Content of overground and underground biomass of *T. angustifolia* in conditions of nitrate nitrogen pollution and with addition of salicylic acid. Note: OP-overgrown part of macrophytes, UP-underground part of macrophytes. 1-control, 2-1 MAC of nitrate nitrogen, 3-10 MAC of nitrate nitrogen, 4-MAC of nitrate nitrogen + salicylic acid, 5-10 MAC of nitrate nitrogen + salicylic acid.

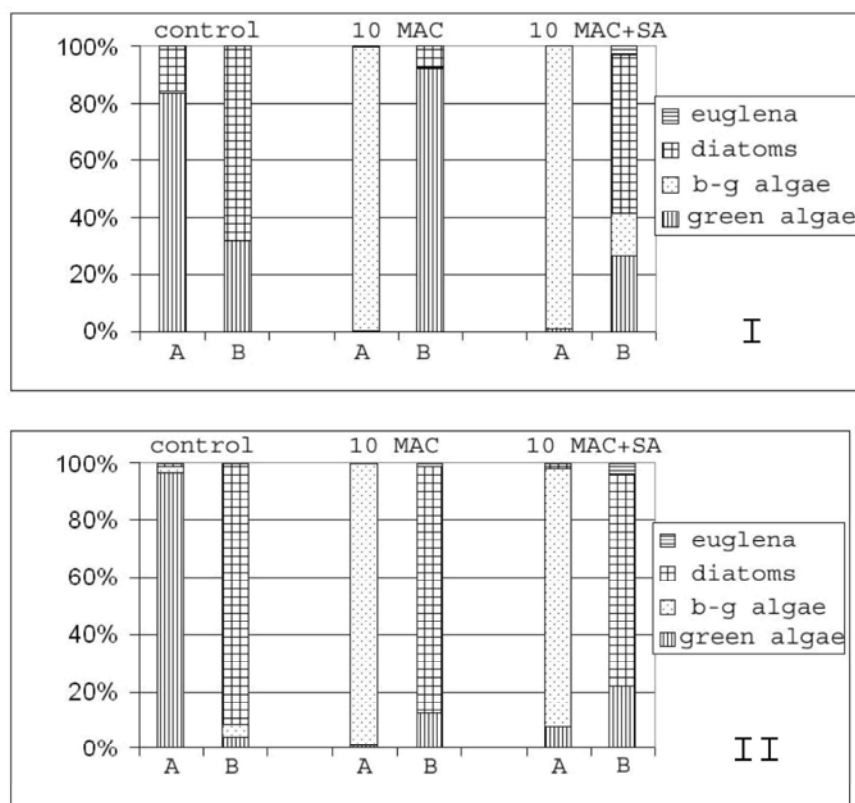


Fig. 2: Proportion of some phytoplankton groups (% from total number): 1 (I) and 2 (II) months since chemical's addition. Note: A-natural water, B-variant with macrophytes; b-g algae-blue-green algae.

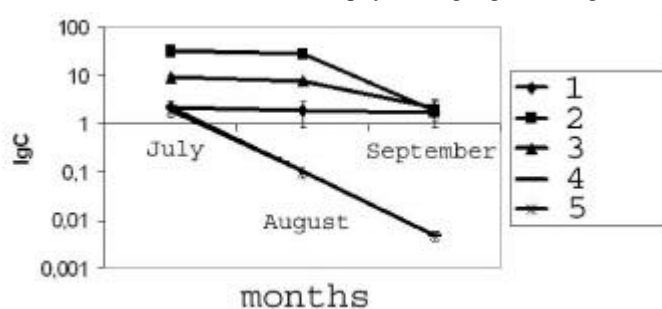


Fig. 3: Seasonal dynamics of nitrate nitrogen content ( $C \text{ mg/L NO}_3^-$ ) in natural water in overgrown biotopes. Note: 1- control, 2-1 MAC, 3-10 MAC, 4-1 MAC + salicylic acid, 5-10 MAC + salicylic acid.

nitrogen concentration was also detected: 1 MAC + salicylic acid-0.005 mg/L vs 1.9 mg/L (control variant); 10 MAC + salicylic acid-0.005 mg/L vs 2.3 mg/L (control variant). The results are presented at Figure 3. This effect was not detected in biotopes without macrophytes. In the end of the vegetation period in variant with salicylic acid, the concentration was 1.9 mg/L vs 1.2 in control, 1 MAC + salicylic acid-136 mg/L vs 46.8 mg/L at 1 MAC, 10 MAC + salicylic acid-663 mg/L vs 620 mg/L at 10 MAC. This suggest on the influence of salicylic acid on hydrochemical regime via activation of metabolism of the

macrophyte. Thus, we detected a mechanism for regulation of hydrochemical regime on nitrate nitrogen by means of its re-distribution in the system "water-macrophyte" toward the latter.

## CONCLUSION

It was stated for the first time that salicylic acid increases effects of regulation concerning phytoplankton and hydrochemical regime. An amount of nitrates decreases thereby stimulating a good quality of natural

water and increasing diatoms. The obtained results provide a new data on mechanisms of systemic resistance of biological objects. It is possible to say now on practical utilization of salicylic acid in the management of structure of aquatic organisms. Also, it is becoming apparent that salicylic acid may be used to regulate water blooming via inhibition of blue-green algae.

#### REFERENCES

1. Enyedi, A.J., N. Yalpani, P. Silverman and I. Raskin, 1992. Signal molecules in systemic plant resistance to pathogens and pests. *Cell*, 70: 879-886
2. Chen, Z., W. Ricigliano and D.F. Klessig, 1993. Purification and characterization of a soluble salicylic acid binding protein from tobacco. *Proc. Natl. Acad. Sci., USA.*, 90: 9533-9537.
3. Szepesi, A., J. Csiszár, K. Gémes, E. Horváth, F. Horváth, M.L. Simon and I. Tari, 2009. Salicylic acid improves acclimation to salt stress by stimulating abscisic aldehyde oxidase activity and abscisic acid accumulation and increases Na<sup>+</sup> content in leaves without toxicity symptoms in *Solanum lycopersicum* L. *J. Plant. Physiol.*, 166: 914-925.
4. Mishra, A. and M.A. Choudhuri, 1999. Effects of salicylic acid on heavy metal-induced membrane degradation mediated by lipoxygenase in rice. *Biol. Plant*, 42: 409-415.
5. Vasiukova, N.I. and O.L. Ozeretskovskaia, 2007. Induced plant resistance and salicylic acid: a review. *Prikl. Biokhim. Mikrobiol.*, 43: 405-411.
6. Yuan, S. and H.H. Lin, 2008. Role of salicylic acid in plant abiotic stress. *Z. Naturforsch. C.*, 63: 313-320.
7. Tarchevskii, I.A., 2001. Pathogen-induced plant proteins. *Prikl. Biokhim. Mikrobiol.*, 37: 517-532.
8. Ratushnyak, A.A., 2002. Ecological and physiological aspects of homeostasis of hydrobiosystems of various organization levels with participation of phytohydrocoens. D.Sc. Thesis, Nizhny Novgorod. In Russian.