Short report

The effect of nitrate nitrogen and salicylic acid on aerenchyma formation in *Typha angustifolia* grown in mesocosms

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Abstract: Macrophytes play a significant role in the functioning of hydroecosystems. Their activities include changes at the physiological and anatomical levels toward the action of various pollutants. This study was intended to reveal some features in aerenchyma formation in roots of *Typha angustifolia* exposed to various concentrations of nitrate nitrogen and salicylic acid. It was found that the resistance of *T. angustifolia* to nitrate nitrogen is mediated by redistribution of aerenchyma formation in the root system. The action of salicylic acids results in decreasing influence of nitrate nitrogen in two types of adventitious roots. Features of aerenchyma formation in the soilborne roots and aquatic ones were different.

Keywords: aerenchyma, marophyte, nitrate nitrogen, salicylic acid, *Typha angustifolia* L.

Introduction

Typha angustifolia L. (cattail) is a widely distributed aquatic macrophyte: in the northern hemisphere, it can be found in the irrigation canals, oxbow lakes, and backwater areas of rivers and streams (Grace et al. 1986). It has the underground thick rhizome and adventitious aquatic and soilborne roots (Gessner 1955). The adventitious roots may be formed also at footstalks and internodes (Rudescu 1974). Macrophytes have a well-developed aerenchyma (Dacey and Klug 1982). Aerenchyma developed in wetland plants like *Typha angustifolia* has crucial functions for plant physiology and also for wetland elemental cycle (Visser and Bögemann 2006). Aerenchymatous tissue, which comprises a high proportion of gas-filled spaces, provides plants with an alternative strategy for obtaining oxygen that is especially important in polluted waters (Drew et al. 2000). Considering the significant role of macrophytes in functioning hydroecosystems (Dhote and Dixit 2009), it seems reasonable to suggest that all organs of the plants as well as airal pathways, providing the gaseous exchange, particularly, in various types of roots, may participate in the detoxication of various pollutants including nitrates. Many authors have proved that the root system with its aerenchyma plays the primary role in the processes of plant adaptation to stress conditions (Demirezen and Aksoy 2004).

Pollution with nitrate nitrogen presents a serious problem for the inland waters (Moore 1991). Therefore, it raises some interest to investigate the anatomical changes in the aquatic macrophyte that occur toward the action of this pollutant. Since the defense action of salicylic acid (SA) was clearly established (Shah 2003), it was interesting to reveal any modulation action of SA to aerenchyma properties under the influence of nitrate nitrogen. Thus, this study was intended to investigate the aerenchymatous changes in roots of *T. angustifolia* exposed to various concentrations of nitrate nitrogen and salicylic acid.

Materials and Methods

Helophyte *Typha angustifolia* was used as object of investigation. Its root system has both thick and thin adventitious roots (aquatic and soilborne ones). The plants have a well-developed aerenchyma that may be detected in all plant organs providing a good gaseous exchange. Investigations were performed at the level of the whole organism.

Investigations were performed during 3 vegetation

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periods (2005-2007) in conditions of mesocosms including natural water (30 L) with the concomitant hydrobionts and curtains of *T. angustifolia* taken from Sredniy Kaban Lake (Kazan, Republic of Tatarstan, Russia) according to recommendations of Xu et al. (1999). It was previously found that volume of 30 L is optimal for reconstitution of the results (Tsirtsis and Karydis 1997). Two types of biotopes were simulated – with and without macrophytes plus natural water. In all biotope, 5 variants with various concentrations of nitrate nitrogen (NN, in the form of $NaNO_3$) (40 mg L^{-1} – 1 maximum allowable concentration, MAC) and salicylic acid (SA, 10^{-4} M) were used according to the following scheme. Biotope with macrophyte: control – natural water without additives; variant 1 – NN (1 MAC); variant 2 – NN (10 MAC); variant 3 – NN (1 MAC) + SA; variant 4 – NN (10 MAC) + SA; variant 5 – SA alone. Biotope without macrophytes contained the same combinations in the variants. The used compounds were introduced to mesocosms 2 weeks after experiment starting (in the beginning of June).

Adventitious roots (aquatic and soilborne ones) were washed with tap water and fixed in 70% ethanol. Transverse sections (6 cm from apex growth) were transferred to glycerin-gelatin mix for analyzing in Biolam-R17 microscope.

Chemical analysis of natural water was performed according to standard methods.

Square of transverse section was calculated on the following formula:

$$S_{ts} = (\pi \times D^2) / 4 \qquad (1)$$

where π is pi number (3.14), D – diameter of transverse section, mm. Square of one air vessel was calculated on the following formula:

$$S_{av} = (\pi \times a \times b) / 4 \qquad (2)$$

where π is pi number (3.14), a and b – length an width of air vessel (in mm). Average square of air vessels was calculated on the following formula:

$$S_a = (S_{av1} + S_{av2} + \ldots S_{avn}) / n \qquad (3)$$

where S_{av1}, S_{av2} and S_{avn} – square of the first, second and n-th air vessels (mm^2), n – a number of air vessels. Proportion (P in percentage) of air vessels was calculated on the following formula:

$$P = (S_{ts} / S_a) \times 100 \qquad (4)$$

Statistical analysis was performed with Statistica 6.0. software. Data is presented as mean ± standard error (n=30). Experimental data were compared using criteria of Student with Bonferroni correction (Hill and Lewicki 2007).

Results and Discussion

It is accepted that living organisms adapt to stress conditions via changes at the physiological and anatomical levels (Sultan 2000). Therefore, investigations of the anatomical rearrangements (in particular, aerenchyma formation) in helophytes seem an important step for understanding the processes of functioning hydroecosystems. Besides, revealing the simultaneous action of pollutant with anti-stress compounds seems an actual task. We selected salicylic acid as anti-stress compound on the basis of previously published data (Rhoads and Mclntosh 1993, Schaller and Oecking 1999).

It was found in this study that nitrate nitrogen and salicylic acid influenced aerenchyma formation in *T. angustifolia.* Table 1 presents data on the proportion of the area of air vessels calculated from the square of transverse section of the roots. The enhancement of air vessels was higher in the aquatic roots compared with those in the soilborne roots (Fig 1 and 2).

The temper of forming air vessels in soilborne vessels depends on seasons. It is interesting to note (Table 1) that the aerenchyma development were more diverse in soilborne roots contrary to aquatic ones considering seasonal dynamics: proportion of air vessels in June and July was 7.2 ± 0.3% and 14.8 ± 1.6%, respectively while the same parameter was 37.2 ± 4.5% and 33.2 ± 3.6% in aquatic roots (Table 1). This is probably connected with features of descending flow of assimilates and different requirements in it among the adventitious roots depending on its functions. Under the action of nitrate nitrogen, aerenchyma formation depended on the type of the adventitious root (aquatic or soilborne), seasons and concentration of the pollutant. In soilborne roots, there was increasing air vessels (but only in June) that favors to enhancement of adaptation to oxygen deficiency. The accumulated oxygen was probably used in vital processes (Sadchikov and Kudryashov 2004). In aquatic roots, we detected decreasing of air vessels under the action of nitrate nitrogen (1 MAC and 10 MAC). This is probably explained by redistribution of the intensity of lysis of parenchyma cells. We detected that salicylic acid influenced the changes in aerenchyma formation. There were increasing and decreasing of air vessels in soilborne and aquatic roots, respectively. Along with nitrate nitrogen (1 MAC), SA activated lysis of parenchyma cells in soilborne roots (the aquatic roots also enhanced their air vessels under NN, 1 MAC + SA condition compared with those under NN, 1 MAC condition.

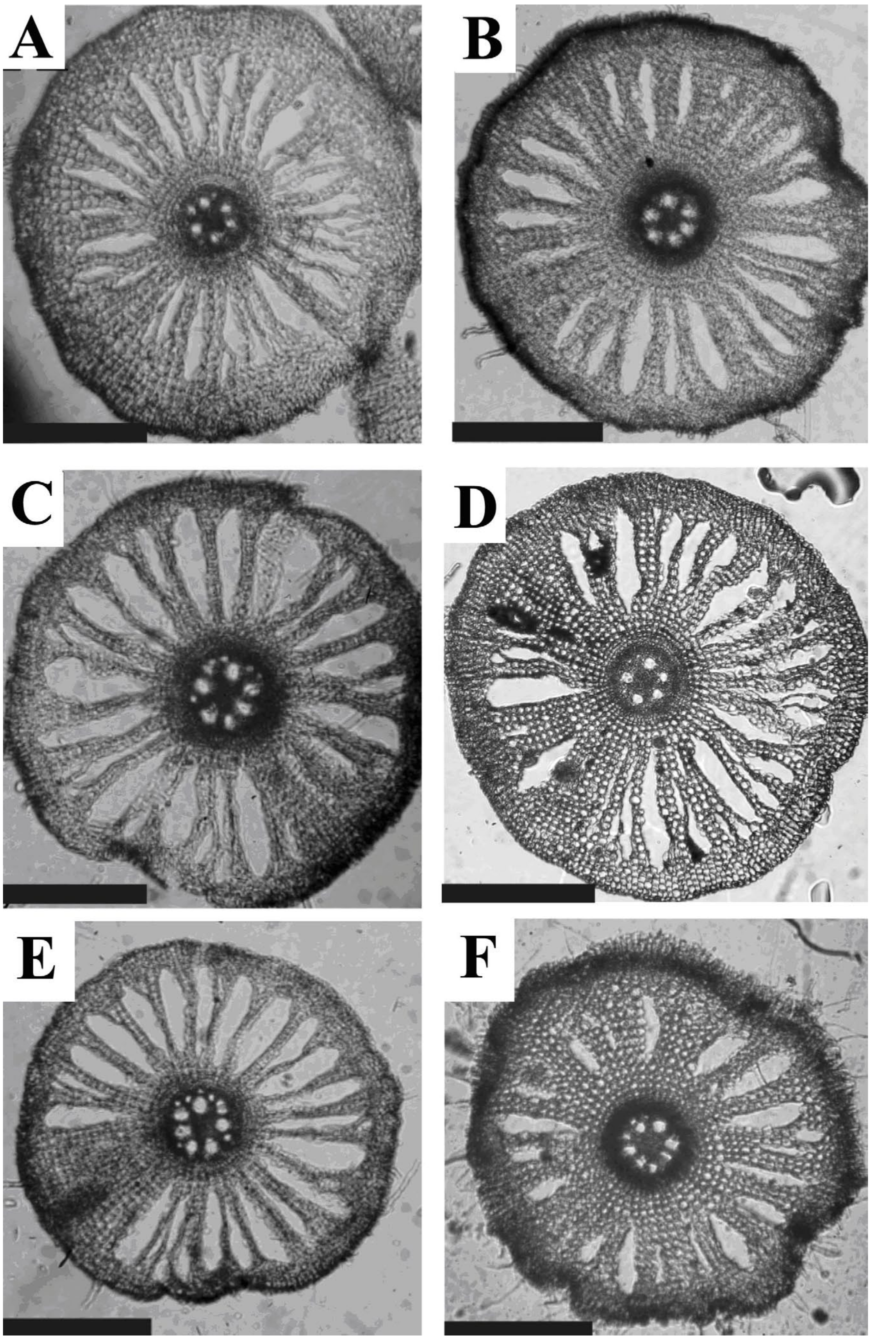


Fig. 1. Transverse sections of adventitious soilborne roots of T. *angustifolia*. Notes: A – control; B – NN (1 MAC); C – NN (10 MAC); D - SA; E – NN (1 MAC) + SA; F - NN (10 MAC) + SA. Bar corresponds to 0.5 mm.

From Table 1, it can be read that both soilborn roots and aquatic roots enhanced their air vessels in July under the NN, 10 MAC +SA condition compared with those under the 10 MAC condition. It is clear from the presented data that the addition of SA results in decreasing harmful action of nitrate nitrogen on soilborne roots – in variant with 10 MAC while on aquatic roots – in variant with 1 MAC only. However, the further experiments are needed to clarify this correlation. Thus, there were adaptation changes in the anatomical structures of *T. angustifolia*. Our results are in agreement of Wolff et al. (2001) that changes in the anatomical features of the adventitious roots toward the action of mineral pollutants may be considered as mechanism of maintenance of their functioning in the environment. Concerning the role of

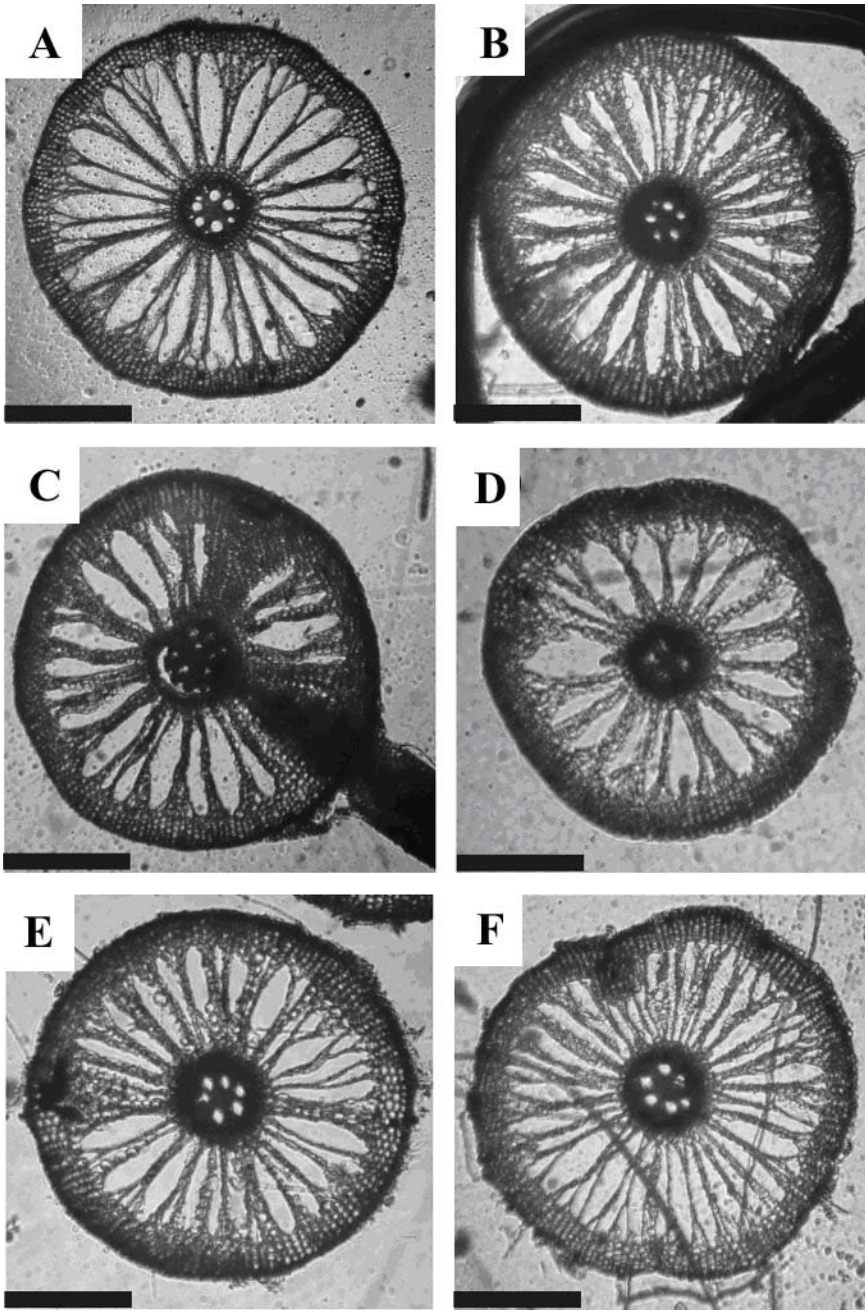


Fig. 2. Transverse sections of adventitious aquatic roots of T. *angustifolia*. Notes: A – control; B – NN (1 MAC); C – NN (10 MAC); D - SA; E – NN (1 MAC) + SA; F - NN (10 MAC) + SA. Bar corresponds to 0.5 mm.

SA, it should be stated that the anti-stress effects of this compound may be quite different at high and low concentrations (Yuan and Lin 2008). It is very likely that other types of phytohormones such as brassinosteroids and jasmonic acid may participate in aerenchyma formation in roots (Wang and Irving 2011).

Conclusions

Resistance of *T. angustifolia* to nitrate nitrogen is mediated by redistribution of aerenchyma in the root system. Increasing concentration of the pollutant results in intensifying this process. The action of salicylic acids results in decreasing influence of nitrate nitrogen in soilborne roots (10 MAC) and in aquatic

Table 1. The proportion of air vessel square (%) in different type of *T. angustifolia* roots under the simultaneous action of nitrate nitrogen and salicylic acid (Mean ± SE)

Variant	Soilborne roots		Aquatic roots	
	June	July	June	July
Control	7.2±0.3	14.8±1.6	37.2±4.5	33.2±3.6
NN, 1 MAC	10.8±0.5 (*6.17, **0.0001)	10.3±1.5 (*2.05, **0.0447)	21.8±2.5 (*2.99, **0.004)	15.2±1.7 (*4.52, **0.0001)
NN, 10 MAC	15.7±0.6 (*14.57, **0.0001)	17.6±1.5 (*1.27, **0.2067)	33.9±3.9 (*0.64, **0.524)	31.4±2.6 (*0.45, **0.6528)
NN, 1 MAC + SA	17.6±1.3 (*7.79, **0.0001)	23.4±1.7 (*3.68, **0.0005)	24.7±2.6 (*2.4, **0.019)	23.9±3.0 (*1.98, **0.051)
NN, 10 MAC + SA	11.9±1.3 (*3.52, **0.0008)	28.5±2.6 (*5.86, **0.0001)	29.1±2.6 (*1.55, **0.129)	37.2±2.8 (*0.85, **0.396)
SA alone	13.2±1.0 (*4.42, **0.0001)	27.2±2.3 (*4.42, **0.0001)	23.4±2.9 (*2.57, **0.012)	32.4±3.1 (*0.16, **0.866)

The values of Student's *t*-criterion (*) for the experimental data (in comparison with control) and the probability level (**) are indicated in brackets.

roots (1 MAC). The opposite direction of aerenchyma formation in two types of roots is probably explained by different adaptation abilities of the organs and distinct environmental conditions where the roots are functioning.

References

Dacey IW, Klug MI 1982 Ventilation by floating leaves in Nuphar. Ame. J. Bot. 69: 999–1003.

Demirezen D, Aksoy A 2004 Accumulation of heavy metals in *Typha angustifolia* (L.) and *Potamogeton pectinatus* (L.) living in Sultan Marsh (Kayseri, Turkey). Chemosphere 56: 685-696.

Dhote S, Dixit S 2009 Water quality improvement through macrophytes - a review. Environ. Monit. Assess. 152: 149-153.

Drew MC, He CJ, Morgan PW 2000 Programmed cell death and aerenchyma formation in roots. Trends Plant Sci. 5: 123-127.

Gessner F 1995. Hydrobotanik. Bd. 1. Energiehaushalt. VEB Deutscher Verlag der Wissenschaften, Berlin, pp. 515.

Hill T, Lewicki P 2007 STATISTICS Methods and Applications. StatSoft, Tulsa, OK.

Moore JW 1991 Inorganic contaminants of surface water: research and monitoring priorities, Springer-Varlag, New York, pp. 334.

Rhoads DM, Mclntosh L 1993 Cytochrome and alternative pathway respiration in tobacco. Plant Physiol. 103: 877–883.

Rodewald-Rudescu L 1974 Das Schilfrohr. Die Binnengewasser, Bd. 27, Scweiterbartsche Verlagbuchhanlung, Stuttgart, pp. 294.

Sadchikov AP, Kudryashov MA 2004 Ecology of littoral plants. Priroda, Moscow, p. 220. (in Russian).

Schaller A, Oecking C 1999 Modulation of plasma membrane H^+-ATPase activity differentially activates wound and pathogen defense responses in tomato plants. Plant Cell 11: 263–272.

Shah J 2003 The salicylic acid loop in plant defense. Curr. Opin. Plant Biol. 6: 365–371.

Sultan SE 2000 Phenotypic plasticity for plant development, function and life history. Trends Plant Sci. 5: 537–542.

Tsirtsis G, Karydis M 1997 Aquatic microcosms: a methodological approach for the quantification of eutrophication processes. Environ. Monit. Assess. 48: 193–215.

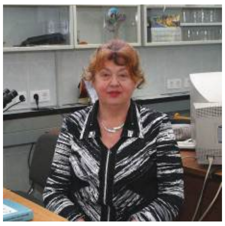
Visser EJ, Bögemann GM 2006 Aerenchyma formation in the wetland plant *Juncus effusus* is independent of ethylene. New Phytol. 171: 305-314.

Wang YH, Irving HR 2011 Developing a model of plant hormone interactions. Plant Signal Behav. 6: 494–500.

Wolff R, Abbott L, Pistorale S 2001 Reproductive strategy of *Bromus catharticus* Vahl (*Cebadilla criolla*): Phenotypic plasticity in natural population progenies. J. Genet. Breed. 55: 67–74.

Xu F-L, Jørgensen SE, Tao S 1999 Ecological indicators for assessing freshwater ecosystem health. Ecol. Model. 116: 77–106.

Yuan S, Lin HH 2008 Role of salicylic acid in plant abiotic stress. Z Naturforsch C 63: 313-320.



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Dr. Andrey Y. Ratushnyak has an interest in the role of adaptogenes in regulation of autoecologic features of autotrophs

Dr. Sergey V. Berdnik has an interest in macrophytes under pollution

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