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Lipoxygenase system under phytohormones and hypoxia in dicotyledonous plants, varietal and interspecific differences

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Motivation and aim: Clarification of lipoxygenase's role in ROS accumulation under hypoxic stress and impact of kinetin and 24-epibrassinolide phytohormones on these processes in pea and soy plants and revealing lipoxygenase functioning in different varieties of soy seedlings under stress.
Materials and methods: 10-day-old seedlings of dicotyledon pea (*Pisum sativum* L.) and soy (*Glycine max* L.) plants hydroponically grown under 12 h photoperiod and +22 °C served as study objects. Aerial part of plant was placed in dark conditions for 3–24 h in different gas media. To investigate impact of kinetin and 24-epibrassinolide, these phytohormones were preliminary inserted by transpiration stream (10 mg/l). LOX activity was determined in tissue homogenate or mitochondria spectrophotometrically by accumulation of hydroperoxides under λ = 234 nm with linoleic acid as a substrate. Enzyme activity was calculated in EU/mg of protein which content was determined by Lowry. Isolation of mitochondria was done by differential centrifugation method. Presence of mitochondrial LOX was determined by native PAGE electrophoresis. LOX localization was conducted by specific development method with presence of hydroperoxides of linoleic acid and cadmium iodide. Experiments were run in 3 biological and 2 chemical repetitions. Obtained data was processed by Excel software package using descriptive statistics method.
Results: Lipoxygenases (EC 1.13.11.12) catalyze oxidation of polyunsaturated higher fatty acids free and bounded in phospholipids with hydroperoxyl derivative formation launching a chain of free radical processes (FRP) in cells. LOX participate in fruit ripening, plant production storage and used in a range of biotechnological processes. Also, LOX play an important role in plant adaptation to stress including hypoxia. Our research showed that LOX activity in soy seedlings (variety "Belgorodskaya 48") was 30% higher compare to pea seedlings (variety "Ramonsky 77"). Under hypoxic stress the LOX activity was increasing in intolerant pea seedlings up to 400% while in more tolerant soy only up to 60% during first 3–6 h of hypoxia and then decreasing in both plants. It was shown that input to hydroperoxide accumulation was given by not only cytoplasmic but also mitochondrial form of LOX which activity was increasing by nearly 80% in first hours of hypoxia in pea seedling and only by 45% in more tolerant soy seedlings and later was decreasing. Abiotic stresses is a main barrier for agriculture development which induce yield decrease. To reduce stress impact the phytohormones acting on levels of metabolic processes and genes activity got involved. Our experiments showed that plant treatment by phytohormone kinetin decreased activity of mitochondrial LOX of pea seedlings in all test variants. Yet in a greater degree it was representative for plants exposed to hypoxic stress when activity of mitochondrial LOX was nearly two times lower. Preprocessing by phytohormone 24-epibrassinolide suppressed LOX activity three-fold in first hours of experiment and by 50% at the end of exposition. Obtained data confirms an important role of phytohormones in plant adaptation processes to hypoxia at a level of plant cells enzyme systems including LOX.
Over the last years was shown that there are not only interspecies but also varietal special aspects of cultivated plants for metabolite content and stress tolerance. However varietal aspects of cultivated plants responsiveness under stress factors are better studied for cereal crops (rice, wheat). Thus, LOX activity in soy seedlings of early-maturing varieties "Belgorodskaya 48", "Pisanka" and "Vyshivanka" recommended for Central region of Russia were analyzed. It was noted that in plants of "Belgorodskaya 48" LOX activity was increasing by 120–175% and in "Vyshivanka" by 215% in first 3–6 h of hypoxia. In soy plants of "Pisanka" no increase of LOX activity under all durations of hypoxic stress was noted. Obtained data was correlated with FRP speed we o *Conclusions* bserved earlier by iron-induced chemiluminescence method for these varieties.
Conclusions: Overall results confirm existence not only species differences but also varietal aspects of soy reactions on FRP and LOX activity process levels which can be used in sort selection processes for specified growth environments. Application of phytohormones kinetin and 24-epibrassinolide allows to affect the speed of ROS formation which is important for tolerance increase to stress for dicotyledon plants and specifically soy due to its acreage growth driven by breeding of northern ecotype varieties.