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# Spermine Induces Autophagy in Plants: Possible Role of NO and Reactive Oxygen Species

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**Abstract**—This is the first study to show that polyamine spermine, a low-molecular-weight nitrogen-containing compound, can induce autophagy in plants. This process is accompanied by an increased generation of reactive oxygen species and nitric oxide, which play a signal role and are required for triggering autophagy.

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Autophagy is a highly conserved process of intracellular degradation of damaged, oxidized, or redundant macromolecules and organelles. Autophagy is involved in the processes of development, growth, stress responses, pathological states, and programmed cell death of eukaryotic organisms. The search for the physiological inducers of autophagy as a defense response for the prevention and removal of toxic effects of stress states is a relevant problem. Such inducers may include polyamines—endogenous low-molecular-weight nitrogen-containing compounds, which are present in various organisms in all compartments and organelles of the cells, including the nucleus, mitochondria, and plastids [1]. The content of polyamines significantly increases under stress conditions, which triggers the defense mechanisms and promotes cell survival [2]. Polyamines exhibit protective properties with respect to high-molecular-weight compounds of the cell, particularly proteins and nucleic acids [3]. There is evidence that exposure to exogenous polyamine spermine (SPM) increased the lifespan of yeast, nematodes, and flies due to the induction of autophagy in cells and that the inactivation of autophagic genes (*ATG*) prevented the life-extending effect [4].

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In plants, polyamines play an important role in the regulation of life activities; in particular, they exert a protective effect under adverse conditions [2, 5]. It was shown [1] that, due to their antioxidant properties, polyamines prevent the death of *Arabidopsis* cells induced by reactive oxygen species (ROS). It is also known that polyamines can induce $H_2O_2$ generation during stress-induced oxidative burst [2] and the formation of nitric oxide (NO) [6]. Previously [7, 8], we recorded an increased formation of autophagosomes in wheat roots under the influence of prooxidants. We found no data on the polyamine-induced autophagy in plant cells in the available literature.

In view of above, the aim of this work was to study the regulation of autophagy in wheat root cells with SPM and the possible role of NO and ROS in this process.

Roots of intact 4-day-old seedlings of spring wheat *Triticum aestivum* L. cultivar Kazanskaya yubileinaya were treated with 10 μM SPM for 3 h. The content of $H_2O_2$ was determined by using xylenol orange [9], and the content of NO was estimated by the EPR signal of mononitrosyl complexes of iron with sodium dithiocarbamate [10]. Visualization of autophagosomes using the LysoTracker Red DND 99 dye (ThermoFisher Scientific, United States) and the assessment of the mitochondrial membrane potential $\Delta\Psi_m$ (tetramethylrhodamine dye) was performed using an LSM-510 META laser confocal microscope (Carl Zeiss, Germany). The ultrastructure of the root central cylinder cells was analyzed using a JEM 1200EX electron microscope (JEOL, Japan). The relative expression level of *ATG* genes was assessed by real-time PCR with the use of specific primers.