Abstract

Leaf senescence is an important physiological process of higher plants and often regarded as the final stage of leaf development. It is highly organised process during which proteins are degraded and nutrients recycled and mobilized to seeds, storage organs or new vegetative growth. Leaf senescence is particularly important for cereal plants. Cereal plants that have late onset and slower rate of leaf senescence have been proven to increase yield. On the other hand, premature senescence induced by stress results in reduced yield and quality in crops. Furthermore, plant senescence can have negative effects on post-harvest storage. For this reason, better understanding of senescence process can have beneficial effects on productivity and quality of grain and the storage life of the harvested tissues. In this thesis I investigated changes in reactive oxygen species level and membrane fluidity (MF) during barley leaf senescence.

Physical properties of thylakoid membranes isolated from barley were investigated by the electron paramagnetic resonance (EPR) spin labeling technique. EPR spectra of stearic acid spin labels 5-SASL and 16-SASL were measured as a function of temperature in secondary barley leaves during natural and dark-induced senescence. Oxygen transport parameter was determined from the power saturation curves of the spin labels obtained in the presence and absence of molecular oxygen at 25 °C. Parameters of EPR spectra of both spin labels showed an increase in the thylakoid membrane fluidity during senescence, in the headgroup area of the membrane, as well as in its interior. The oxygen transport parameter also increased with age of barley, indicating easier diffusion of oxygen within the membrane and its higher fluidity. The data are consistent with age-related changes of the spin label parameters obtained directly by EPR spectroscopy. Changes in the membrane fluidity of barley secondary leaves were compared with changes in the levels of carotenoids, proteins and polyisoprenoid alcohols (PA) which are known to modify membrane fluidity. Determination of total carotenoids and proteins showed linear decrease in their level with senescence, while the level of PA increased. The results indicate that thylakoid membrane fluidity of barley leaves increases with senescence; the changes are accompanied with a decrease in the content of carotenoids and proteins, and increase in the level of PA which could be a contributing factor. Fluidization of the membrane, allows for better penetration of the oxygen inside the membrane, which can lead to increase in the production of ROS. Production of ROS could be further facilitated by a decrease in the activity of xanthophyll cycle. It was shown that access of violaxanthin de-epoxidase to its substrate, violaxanthin, depends on MF. Since carotenoids (car) of the xanthophyll cycle protect plants against excess of light and oxidative stress, a decrease in activity of the cycle may lead to oxidative damage to the thylakoid membrane and an increase of ROS production. Indeed, the production of ROS started to increase together with observed fluidization of the membrane from 22 to 29 day after sowing (DAS). Thereafter, production of ROS started to decline till 35 DAS. Finally, on the last day of the measurement, 39 DAS, senescence is entering the last phase; chl is at 25 % of its initial value, level of lipid peroxidation products reaches the highest value and H_2O_2 increases again which contributes to the final degradation of the cell structure.