2.2. Response of the photosynthetic apparatus to low temperatures: photodamage

- **ROS as the main damaging factor in the conditions of cold stress**

ROS are highly reactive forms of oxygen and they accumulate after different types of stress (biotic or abiotic), thus damaging the components of the cell with their action: they oxidize the lipids, damage the DNA and have a negative impact on the physiological process in the cell. ROS form mostly in the chloroplasts, mitochondria and peroxisomes. Mitochondria do not take so big part in ROS formation in plant cells, as compared with animals, probably because of the alternative oxidase enzyme (AOX), which competes for the substrate of the cytochrome bc_1 complex (Purvis, 1997; Apel and Hirt, 2004). There is a balance between the formation and degradation (neutralization) of ROS in the normally functioning cell. However, when the organism is a subject of stress, this balance is disturbed and ROS start to form inside the organism.

Regardless of the highly thermodynamic reacting ability of the molecular oxygen (O_2), reactions, performed with it require very high activation energy, because of the existence of free π electrons with parallel spin in the main triplet state of the molecule. According to Pauli's principle, electrons with antiparallel spins only can form a couple. For a reaction with oxygen to take part, one of the two free π electrons has to acquire different spin, for which a certain amount of energy in needed. In specific conditions (stress, high light intensity) however, in the reaction centre of PS II and the antennae complexes as well, a triplet chlorophyll forms, that is capable of producing singlet oxygen ($^1O_2$) (Fig. 1). It can be reduced more easily than triplet oxygen and can produce ROS that can bring serious damage to the plant cell, if they are not neutralized. $^1O_2$ can easily react with proteins, lipids (and mostly with the polyunsaturated fatty acids), pigments (pigment bleaching), and to deactivate PS II by degradation of the protein D_1 (Prasil et al., 1992; Aro et al., 1993). Furthermore, when enough energy is available, oxygen can be reduced directly to a superoxide ion ($O_2^{-\bullet}$) and after that to a peroxide ($O_2^{-2\bullet}$), accepting electron at each of the subsequent steps in the process of ROS formation. After that, these ROS can be protonated until a peroxy radical, hydrogen peroxide and water are formed (Fig. 2). A detailed scheme of the formation and reduction to water of ROS in the chloroplast and peroxisome is given in Fig. 2.

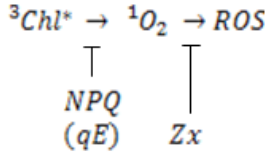

Fig. 1 The main pathway of generation and inhibition of ROS inside PS II. Zx – zeaxanthin (bound to the membrane or in a complex with LHCII) functions synergistically with the tocopherols; qE – non-photochemical quenching (NPQ) with the main participation of PsbS, the first and the fastest phase from the NPQ

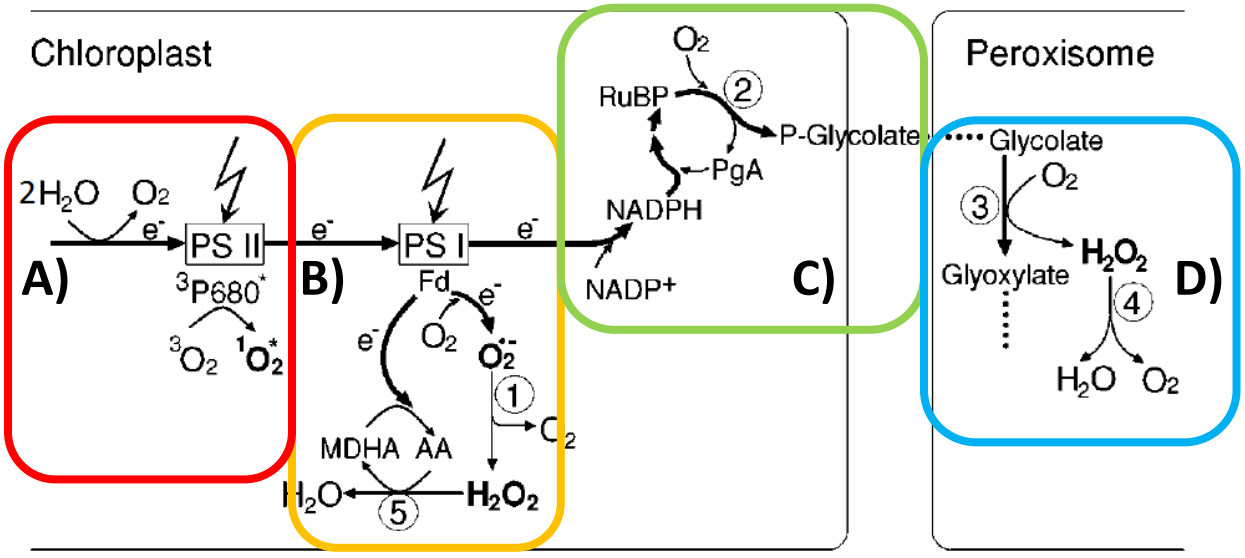

Fig. 2 Processes of ROS generation and their reduction to water in the chloroplast and the peroxisome. A) Singlet oxygen formation ($^1O_2^*$) in PS II. $^3P680^*$ – triplet state of the chlorophyll molecules in the reaction centre of PS II. $^3O_2$ – triplet state of oxygen; B) Photoreduction of oxygen to superoxide radical ($O_2^{\cdot-}$) by the FeS cluster of PS I. The superoxide radical is reduced to hydrogen peroxide with the help of the enzyme superoxide dismutase (1). After that the hydrogen peroxide is reduced to water by the enzyme ascorbate peroxydase. In the process a molecule of ascorbate (AA) oxidises to monodehydroascorbate (MDHA); C) Photorespiration – oxygenase reaction of Rubisco (2). PgA - phosphoglycerate; D) Deactivation of the hydrogen peroxide to water and oxygen by the enzyme catalase (4) in peroxysomes. (Apel and Hirt, 2004).

After the so-called photooxidative damage in plants very large amounts of ROS are produced. When plants are in conditions of light intensity, higher than their capability for assimilation of CO_2, an overreduction (absorbance of electrons) of their electron-transporting components occurs. The latter leads to inactivation of PS II and inhibition of the photosynthesis because of the accumulation of the forementioned ROS. A detailed view shows, that in the process of photooxidative damage the unused additional energy leads to the formation of singlet oxygen from PS II and superoxide ions (radicals) of oxygen from PS I (Fig. 2 A; B) (Asada, 1999; Krieger-Liszkay, 2004). Plants that have adaptation abilities for growth and development in abiotic stress conditions are often a subject to the consequences of the photooxidative damage. These plants tolerate better this type of damage, mainly because of their ability for quenching and neutralization of ROS (Oquist and Huner, 2003; Apel and Hirt, 2004).

The first way of dealing with ROS is diminishing the possibility of their production. This takes place when processes are being activated, that consume the excess oxygen even before it turns into ROS. Three processes assimilate the oxygen inside chloroplasts: I) the oxygenase reaction of Rubisco (or the so-called photorespiration, which is the basic process, assimilating the excess oxygen inside chloroplasts) (Fig. 2 C); II) the direct reduction by PS I (photoreduction – that occupies up to 30% of the electron transport in conditions of intense light) (Fig. 2 B). The next mechanism for suppression the action of ROS at the place of their formation is the quenching of the triplet chlorophyll forms in the content of PS II. The triplet chlorophyll forms, which are a structural part of the antennae complexes are denoted as ^{3}Chl and $^3P_{680}$* is the triplet chlorophyll from the reaction centre of PS II. $^3P_{680}$* can form in two ways, firstly by change in the spin of the electron from singlet chlorophyll and secondly by reactions of charge recombination in PS II (Krieger-Liszkay, 2004). The former mean for formation of triplet chlorophyll (^{3}Chl) is unique inside the antennae complexes, whereas the latter is the main process, concerning the formation of $^3P_{680}$* inside the reaction centre of PS II. Charge recombination normally occurs at the stage of the photochemical reactions from the light phase of photosynthesis. The trapped quantum of light energy from the reaction centre of PS II (P_{680}) is used up in the process of separation and accumulation of electric charge in the components of the electron-transporting chain, and after that is utilised to create chemical potential from both sides of the thylakoid membrane.

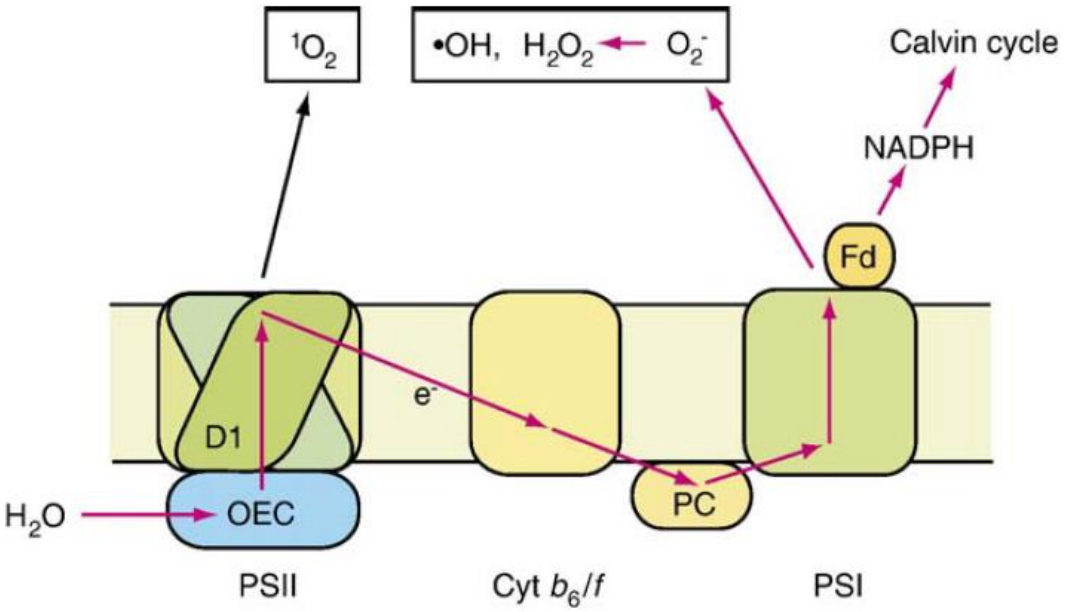

Fig. 3 Diagram for the production of ROS in both photosystems. The transfer of energy from chlorophyll in triplet state to the molecule of oxygen leads to formation of a singlet oxygen molecule ($^1O_2$). The reduction of oxygen from the acceptor side of PS I, as a result from photosynthetic electron transport leads to production of superoxide ion (O_2^-), which transforms afterwards to hydrogen peroxide (H_2O_2) and OH. Formation of ROS is induced by strong light and a decrease in the fixation of CO_2. Red arrows represent transfer of electrons, and the black ones are transfer of energy. Fd, Ferredoxin; OEC, the oxygen-evolving centre; PC, plastocyanin. (Nishiyama et al., 2006)

This occurs because of the property of the chlorophyll molecule from the reaction centre to absorb a quantum of light, switching itself into a higher energetic state of a singlet chlorophyll form (P_{680}*). During this process one of the coupled electrons from the porphyrin ring "jumps" to an orbital with higher energy. This state of the chlorophyll molecule lasts for about several nanoseconds, which is enough for reaction of charge separation between the components of PS II to be carried out; more specifically, the chlorophyll from the reaction centre, pheophytin and finally, the primary quinone acceptor (P_{680}*Pheo –> $P_{680}^+Pheo^-$ –> $P_{680}^+Q_A^-$). After the charge separation between the reaction centre and Q_A there is a probability for the reverse recombination to pheophytin to occur. Since the state $P_{680}^+Q_A^-$ is relatively continuous, a high probability exists that the electron, which came back to $P_{680}^+Pheo^-$ to have changed its spin and then the formation of triplet state $^3[P_{680}^+Pheo^-]$ could be possible (Durrant et al., 1990). Several factors facilitate this process, mostly the change of redox potential of Q_A after stress, whereas some herbicides like DCMU and phenol photosynthetic inhibitors

affect it positively or negatively (Keren et al., 1995, 1997; Nakajima et al., 1996). The overreduction of the photooxidative components, followed by blockage of the photochemical reactions also increases the possibility for charge recombination and formation of $^3P_{680}$. For example the accumulation of plastoquinone in its reduced form can obstruct the movement of electrons further away, which creates favourable conditions for recombination (return) of the charge to the reaction centre of P_{680} (Krieger-Liszkay, 2004).

Plants possess mechanisms for neutralising of the already created triplet forms of chlorophyll, as well as singlet oxygen. Carotenoids, associated with the antennae complexes can directly quench ^{3}Chl and $^1O_2$, forming triplet carotenoid, which dissipates the energy in the form of heat. However, this can only occur when carotenoid molecules are in a direct contact with ^{3}Chl and $^1O_2$. If the space between them is higher than the Van der Waals distance (3.6 Å), the electron orbitals cannot overlap and neutralization of active forms of chlorophyll and oxygen cannot occur. Inside the reaction centre of PS II there are two molecules β-carotene, but nevertheless, the distance between them and the reaction centre is kept too large because of the high oxidative ability of P_{680}^+. This fact represents an obstacle for the direct deactivation of triplet chlorophyll inside the reaction centre. Perhaps only $^1O_2$ could be neutralised by the carotenoids from the PS II (Telfer, 2002). Therefore, only the direct quenching of $^1O_2$ can be realized there, mainly with the active participation of the tocopherols (Trebst et al., 2002, 2003; Kruk et al., 2005). If $^1O_2$ is not neutralized, it induces the enzymatic degradation of the core protein D_1, which is one of the two main components of PS II and this leads to inhibition of photosynthesis (Fig. 4). The degradation of D_1 inhibits the function of P_{680}, including the formation of $^1O_2$. This is considered to be an effective system for the deactivation of the $^1O_2$ itself (Trebst, 2003). Lately, the damaged and degraded D_1 protein is synthesized *de novo* and PS II is restored in the cycle of damage and repair of D_1 (Prasil et al., 1992; Aro et al., 1993) (Fig. 4). Reactive oxygen species, mainly superoxide ions can also form in PS I (Fig. 3) (Apel and Hirt, 2004; Nishiyama et al., 2006). This occurs at the ferredoxin acceptor of PS I, which is a very strong reductor and can easily reduce the oxygen molecule. The reduction of oxygen in PS I is a concurrent process to the reduction of $NADP^+$. In stress conditions, when the fixation of CO_2 is low and not enough amount of NADPH is utilized, the reduction of oxygen from PS I can be a reason for the formation of reactive oxygen species (Szarka et al., 2012). The superoxide radicals, produced in PS I are deactivated mostly from the enzymatic system of plants, including the enzymes superoxide dismutase and ascorbate peroxidase (Asada, 1999).

In conditions of oxidative stress, plants activate their protection mechanisms against accumulation of ROS. These mechanisms include the increase of the antioxidant activity in an enzymatic or non-enzymatic way. The first type of antioxidant protection includes the enzymes superoxide dismutase, which deactivates the superoxide ions, the ascorbate peroxidase, deactivating the hydrogen peroxide by the means of the ascorbate as a cofactor and the enzyme from the peroxisomes catalase, that also deactivates the hydrogen peroxide, formed inside the peroxisome (Apel and Hirt, 2004). Non-enzymatic antioxidant defence is realized by the increased synthesis of antioxidant molecules: tocopherol, ascorbate, glutathione (Szarka et al., 2012). The other defence mechanisms of photosynthesis include the non-photochemical quenching and the xanthophyll cycle (Jahn et al., 2012). The non-photochemical quenching (NPQ) appears to be the main protective mechanism against the oxidative stress as a result from the excessive light energy (Krause and Weis, 1991). NPQ can be divided to several processes or stages in relation to the period of their activation and the duration of their action (Lambrev et al., 2010; Nilkens et al., 2010). qE (from **e**nergy dependent **q**uenching) denotes the quickest stage (Krause et al., 1982) and includes the activation of the dissipation of excessive energy from the protein PsbS (Li et al., 2000). This stage activates during several seconds and it depends on the accumulation of the proton gradient (ΔpH), thus it is also called ΔpH dependent NPQ (Li et al., 2002; Li et al., 2004).

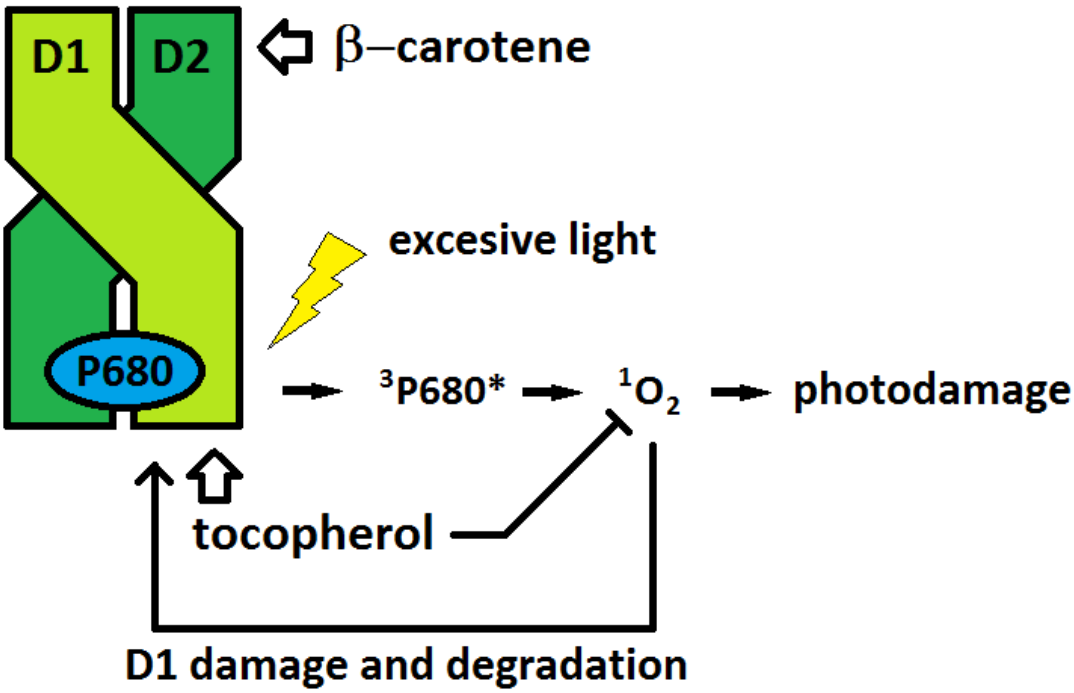

Fig. 4 Diagram of photoinhibition of PS II; formation of reactive oxygen species and the involvement of tocopherol in photoprotection of D1. Carotenoids do not participate directly in the photoprotection of D1, but this is realized by tocopherol. They quench the singlet oxygen, which is a reason for damage and activation of a cycle from the enzymatic degradation and repair of the protein D1. Singlet oxygen is molecule with high reactivity, capable of oxidation of many other components of the photosynthetic apparatus, the unsaturated fatty acids from the membranes being oxidised most strongly. (Wide arrows show the location of the molecules of β-carotene / tocopherol in PS II)