2. LITERATURE REVIEW

2.1. Common characteristics of the low temperature stress. Physiological and molecular levels of effect.

The definition of stress is every external factor, exerting unfavourable effects on plants. Two types of stress are known depending on factors, leading to them: biotic and abiotic. The biotic stress is caused by biologic agents, called pathogens (viroides, viruses, bacteria, fungi, herbivores, parasitic plants). The abiotic stress includes factors from the environment such as high and low temperatures, drought, salinity stress (high concentration of NaCl or other salts), mineral deficit, hypoxia and anoxia (after hyperhydration of the roots, flooding). Cold stress can be two types, as well: *chilling* stress (low positive temperatures) and *freezing* stress (temperatures below 0^{o}C). Based on their way of dealing with low temperatures there are three groups of plants. Plants from the first group are able to survive the effects of low temperatures by the means of certain anatomical, physiological and biochemical characteristics. Plants from the next group can survive brief periods of exposure to stress from low temperatures. The third group of plants evade the stress periods in the form of seeds in the case of the annual plants, or underground parts (tubers, roots etc.) in the case of the perennial plants (Taiz and Zeiger, 2006).

It is well-known, that the main target of low temperatures are the biological membranes. The latter lose their liquidity and can be more easily destroyed in cold stress conditions, especially when they lack enough polyunsaturated fatty acids (Williams et al., 1988). This leads to leakage of the water content out of the cell, as well as valuable for the cell substances, together with the water. When the concentration of polyunsaturated fatty acids in the cell membranes is higher, the cell can survive lower temperatures. Since the polyunsaturated fatty acids have lower melting point and exist in liquid state at lower temperatures, their presence inside the cell is a requirement for its higher tolerance to cold stress by retaining the elasticity of the membranes (Palta et al., 1983).

Proteins are also influenced by the low temperatures, as they lose their hydration shell and their hydrogen bonds break, so that the proteins shrink and their denaturation is easier. After sufficiently strong induction with low temperatures on the proteins of the cell, their cysteine groups bind irreversibly, forming disulphide bridges and thus they cannot restore their native form after rising of the temperature. As a consequence, the proteins cease to function normally. Being an important part of the cell membranes (with the permeability as their main function), the denaturation of proteins also exerts a dehydration effect on the cell. Besides that, the function of proteins as enzymes makes them an important part of almost every biological process and their impairment affects significantly the intensity of the life functions of the cell (Taiz and Zeiger, 2006).

Other very important aspect of the stress in conditions of low temperatures is the ice formation and damages caused by it. After the temperature of plant tissues drops below 0^{o}C, the water inside them starts turning into ice, which does not occur instantly. Initially, the temperature decreases quickly few degrees below zero, without formation of ice. This process is known as supercooling. Immediately after that in the apoplastic system ice starts to form, and simultaneously temperature rises and stays around zero, since a process of releasing the latent heat occurs. After most of apoplast water has turned into ice, temperature starts to decrease again, but this time ice forms inside the cells, as well (Brown et al. 1974).

Low temperatures affect membranes of the cell, mostly the thylakoids as well as proteins, thus they stop the growth of plants and decrease or completely inhibit the photosynthetic activity. Inside cells of tolerant to cold temperatures plants, polyunsaturated fatty acids are synthesized, sustaining the stability and the liquid state of cell membranes at lower temperatures (Nishida and Murata, 1996). Besides that, low temperatures inhibit absorption of water and mineral nutrients from the roots, which in combination with the water leakage from the cells is a reason for overall dehydration of the plant.

When cold stress is exerted in the presence of enough light, plant cells suffer from photodamage as well (Takahashi and Murata, 2008). The light-harvesting complexes and photosystems are still intact then, but the fixation of CO_2 is lower because of the lower speed of the biochemical reactions from the Calvin cycle and the electron acceptor $NADP^+$ depletes. This causes electrons, donated from photosystems, instead of being transported for $NADP^+$ reduction to bind with oxygen and thus to form ROS, which damage the cell (Wise, 1995). Therefore, evergreen plants from the moderate, arctic and alpine areas decrease the amount of their light-harvesting complexes during winter. Moreover, the protein PsbS / CP22 (a peripheral protein form PS II, associated with LHCII) turning excess unused light energy in the form of heat, accumulates in higher concentration. The energy, absorbed from the LHC of PS I, is used for carrying out work on the principle of the cyclic phosphorylation, as well (Oquist and Huner, 2003). Besides the changes on the level of both photosystems, the cells of plants, tolerant to low negative temperatures synthesize also cryoprotectors, like sugars, proline etc., that are osmotically strong compounds and can bind free water, thus lowering the freezing point of water and stabilizing the proteins (Dexter, 1933; Anderson, 1944; Sakai, 1962; Levitt, 1980). Apart from that, especially in tree species and plants, tolerant to very low negative temperatures, are synthesized the so-called antifreeze proteins, that obstruct the nucleation of ice crystals, thus enabling plants to survive very low temperatures (Griffith et al., 1997). The process, known as cold acclimation is of extreme importance for most cold tolerant plants for their survival at low temperatures (Weiser, 1970). They acquire resistance by inducing dormancy and dehydration of the cell, because the formation of ice crystals is reduced when the content of free water is low. ABA is the main signal for these plants to enter the phase of acclimation (Mantyla et al., 1995). As a stress hormone, ABA is synthesized and accumulated in higher amounts after treating with low temperatures. Some plant species are able to withstand up to -40ºC after acclimation with low (chilling) temperatures or exogenic treatment with ABA (Gusta et al., 1996).