

8. Abstract

The geographical distribution of plant species is limited by various environmental factors, two of which are probably the most important - the water availability and temperature. In addition, these two phenomena are closely correlative whereas increased hardiness to one leads to increased hardiness to the other. Perhaps the most feared stress factor is the low temperature. The perception and sensibility are very variable among plants, so we can distinguish the species as chilling or freezing sensitive, insensitive or tolerant. The ways of managing the environmental stress are as various as is the plant kingdom itself. The same variety can be observed even in the scope of one plant - some of plant's parts try to avoid the stress, other tolerate it to some measure. Besides defence mechanisms developed during evolution, plants are also capable of much faster adaptation to current conditions by acclimation. However, even this process requires enough time to develop the maximum tolerance possible.

Chilling and freezing tolerance of many plants is supported by accumulation of osmotically active agents called compatible solutes, among which one of the most spread is the amino acid proline. Thanks to its amphiphilic nature, it is able to hold back the water within the cells under conditions of osmotic stress. It can also stabilize membrane and cytoplasmic proteins as well as membranes themselves. The metabolism of proline *per se* is of the same importance – because of the regulation of synthesis and degradation of this amino acid it comes to cycle process between proline and its intermediates under various conditions. This is the way, how the redox potential can change (the enzymes of proline biosynthesis require NADPH as cofactor). Proline can be the means of reducing acidity of cytoplasm and the compound able to scavenge reactive oxygen species. After recovery from stress, proline becomes the source of carbon, nitrogen, energy and reducing equivalents, which makes the recovery of plant's normal metabolism easier.

Because of the proline's participation in the stress tolerance, the aim of this thesis was to find out if it is possible to obtain winter oilseed rape plants with increased level of proline and freezing tolerance using *in vitro* selection in microspore cultures by *trans*-4-hydroxy-L-proline. The partial aims were the selection of hydroxyproline resistant (Hyp-resistant) regenerants, the determination of the proline content in the respective variants (Hyp-resistant and control) and the comparison of the values of control plants and Hyp-resistant regenerants. The goal was also to find out the

relationship between the grade of reached freezing resistance and the level of proline in leaves of control and Hyp-resistant plants.

By microspore embryogenesis and *in vitro* selection 52 Hyp-resistant regenerated plants were obtained. As for regenerants of F₆ donor plants, there was no significantly higher content of proline in the leaves of Hyp-resistant than in control doubled haploid plants. The cold hardiness was significantly higher in HP – 10A variant if compared with the control plant. Although some Hyp-resistant doubled haploids (CH993 – 10G, CH2008 – 10D) obtained from hybrid donor plants showed higher levels of proline in leaves than control plants, differences between Hyp-resistant regenerants and the respective control plants weren't in most cases significant. Only in one variant resistant to toxic analogue (OP25 – 10) lower level of proline was found. Significantly higher cold hardiness than in control plants was reached in case of two doubled haploid Hyp-resistant plants – OP24 – 10A2 and OP24 – 10BX. In the rest of regenerants resistant to hydroxyproline hardiness wasn't either statistically important or it was lower than the hardiness of control plants. The double haploid Hyp-resistant regenerant of OP21 genotype exposed after two months' acclimation by 4°C to temperature shock (-7°C) for 3,5 hours, showed significantly higher level of cold hardiness than the control plant treated same way. The constitutive proline level in the leaves of the mentioned Hyp-resistant regenerant after acclimation reached double values if compared with the control plant. In the *in vitro* plants, acclimated for 16 days, the variability was observed – not only in the amount of proline in the leaves but also in the leaves' dry weight. The amount of proline in the leaves of *in vitro* plants and the percentage of dry weight of the same leaves didn't correlate. A slight dependence of cold hardiness on the proline content in the doubled haploid plants' leaves was found. Less than 13% of their cold hardiness was possible to explain by the proline content in the leaves of tested plants.

With respect to conclusions mentioned above, for the application of the winter oilseed rape's *in vitro* selection with increased freezing tolerance, it could be appropriate to use mutagenic agents for raising the natural variability of F₁ hybrid plants' microspores. The freezing tolerance could be given by other factors than by constitutively increased level of proline and on the other hand, proline can contribute to tolerance of different stress factors with the same determinant – osmotic stress.