

Summary

Aluminum is the third most abundant element in the earth's crust. It is toxic in acidic soils. At low pH, aluminum is genotoxic, causing DNA damage and cell cycle arrest. Barley is one of the most sensitive cereals to aluminum stress. Its effect is manifested in the limitation of plant growth and development, and consequently in lower barley yield. Inhibition of root growth is a well-known indicator in response to aluminum stress. In addition to pH, many factors influence the toxicity of aluminum, including the type of medium (under laboratory conditions), the concentration of aluminum, and the exposure time.

The doctoral thesis aimed to evaluate the effects of aluminum in the root cells of barley, the cultivar Sebastian and the *hvatr.g* mutant (Ataxia Telangiectasia and RAD3 Related, ATR), with increased tolerance to aluminum, with particular emphasis on DNA damage and cell cycle disturbances. Due to the influence of aluminum on root growth, the analysis of histological and anatomical changes in the roots and the composition of the cell wall was also performed.

It has been shown that aluminum causes genotoxic effects in barley cells of the cultivar Sebastian - the formation of micronuclei, cell nuclei damage, and direct DNA damage in the form of SSB and DSB detected in the TUNEL test.

To assess the effect of aluminum on the cell cycle, flow cytometry and the incorporation and detection of the EdU thymidine analog were used. In addition to a decrease in the mitotic activity of root cells, the changes in the cell cycle such as the reduction in the frequency of cell nuclei in the S phase and an increase in their frequency in the G2/M phase were observed.

Histological analysis of the roots of the cultivar Sebastian and mutant *hvatr.g* grown in media with different pH and with the addition of aluminum chloride showed differences in the anatomy of the roots. Among them, an increase in the root diameter and the size of rhizodermal cells, a shortening of the root cap, and cell divisions of the exoderm and cortex were observed.

The analysis of the presence and distribution of pectin epitopes, recognized by the antibodies LM5, LM6, LM19 and LM20, showed differences in the roots of the Sebastian cultivar: control and treated with aluminum chloride. The cell response to aluminum was expressed by the specific distribution of pectins in the cell wall and thus allows the knowledge of aluminum toxicity to be improved by elucidating the root growth inhibition mechanism. Moreover, it has been shown that methyl esterified HG can be a marker of newly formed cell

walls under aluminum stress. Histological changes were observed in the roots of the parental cultivar in control at pH 6, pH 4, and with the addition of aluminum chloride.

Using the *hvatr.g* mutant allowed to describe the role of ATR in the DDR pathway in barley cells and the function of the *HvATR* gene in response to DNA damage caused by aluminum toxicity. The analysis of the possible role of the ATR-dependent DDR pathway in response to another DNA damaging factor, the chemical clastogen maleic hydrazide (MH), was also undertaken. The meristematic root cells of the *hvatr.g* mutant were characterized by a high level of DNA damage under control conditions which may indicate a disturbed DDR pathway. The high frequency of DNA damage did not lead to cell cycle arrest, but the mutant showed an increased frequency of cells in the G2/M phase. In response to treatment with aluminum chloride and MH, mutant *hvatr.g* showed a high level of tolerance – there was no inhibition of root growth that was observed as in the parental cultivar.

Moreover, treatment with aluminum chloride and MH increased the level of DNA damage but did not affect the mitotic activity and cell cycle profile in *hvatr.g* mutant cells. Studies of the effect of aluminum and MH in barley *hvatr.g* mutant cells with a damaged DNA repair pathway and its parental cultivar, Sebastian, confirmed the *HvATR* gene's role in the mechanism of the response to the used factors. Impaired DNA damage response mechanism may result in tolerance to aluminum and MH.