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Global Toxicology 2020: Understanding of the aluminum cyto- and genotoxicity in Hordeum vulgare roots with an emphasis on DNA integrity and cell cycle using cyto-molecular approaches- Jolanta Kwasniewska- University of Silesia

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Aluminum (Al) is one of the most important crust elements causing reduced plant production in acidic soils. Barley is one of the cereals that are most sensitive to Al. Al in acid soils limits barley growth and development and, as a result, its productivity. Since the mechanism of Al toxicity is discussed we cytogenetically explored the genotoxic consequences of Al on the barley nuclear genome. For Al-genotoxicity testing the following parameters were analysed: mitotic activity, cell cycle profile and DNA integrity. We demonstrated the cytotoxic and genotoxic effects of Al in barley root cells. Al treatment significantly reduced the mitotic activity of the root tip cells and it also induced micronuclei and damaged nuclei. The DNA-damaging effect of Al was observed using the TUNEL test. We defined the inhibitory influence of Al on DNA replication in barley. Analysis with the labelling and detection of 5-ethynyl-2‘-deoxyuridine showed that the treatment with Al significantly decreased the frequency of S phase cells. We also demonstrated that Al exposure led to changes in the cell cycle profile of barley root tips. The delay of cell divisions observed as increased frequency of cells in G2/M phase after Al treatment was reported using flow cytometry. We demonstrated that Al-dependent DNA damage is in large part responsible for root growth inhibition following exposure to Al. An extended view of the genotoxic consequences caused by Al toxicity greatly improved our understanding of these processes.

‘Sebastian’ to Al compared to other species. The mechanism that is responsible for the decreased cell division rate in roots after Al treatment may be connected to the direct Al bind- ing to the DNA phosphate backbone [37,38]. The cytogenetic effects of Al treatment in barley that was observed in the presented study were compatible with the changes in the root growth parameters, such as decrease in the total root length, total root area and total root volume.

Detailed analyses of the symptoms of root growth inhibition after Al treatment, which have not previously been reported for barley, were possible using a specialized root scanner coupled with the WinRHIZO software. These analyses are easy to handle and quick and therefore pro- vide valuable data to predict the cytogenetic effects that are responsible for root inhibition, thereby replacing the time-consuming analyses in Al-optimizing experiments.

The impact of Al on DNA has already been suggested [38]. The effects of Al3+ ions on DNA integrity, which are observed as micronuclei, have been demonstrated in many species. Minet al. [39] reported a significant increase in the frequency of micronuclei in Vicia faba root tip cells after Al treatment in the range 0.01–10 mM. Chromosome aberrations induced by Al have also been reported in wheat [40] and rice [41]. Our results demonstrate that Al is a weak clastogenic agent in Hordeum vulgare cultivar ‘Sebastian’ cells that are exposed to the tested Al concentrations in a range of 20–40 μM. We also found that Al disturbed the morphology of nuclei, which has not previously been reported. This effect may be one of the symptoms of cell death that is induced by Al. The studies of Pan et al. [13] described some aspects of pro- grammed cell death (PCD) and suggested that Al can lead to this process in barley and other plant species. Al-induced cell death has been studied in six cereal species including maize, wheat, triticale, rye, barley and oat [42]. DNA fragmentation, which was analyzed electropho- retically and indicated PCD, was observed in rye, barley and oat roots, but not in maize and wheat. These results suggest that wheat and maize are more tolerant to Al than the other ana- lyzed species [42]. Data from our study using the TUNEL test confirmed that Al treatment induced DNA fragmentation in the barley root tip cells and therefore support this theory about PCD. The frequency of positively labeled nuclei in the TUNEL test was significantly dif- ferent from the control only after treatment with 40 μM Al. As TUNEL-positive cells occurred more frequently than the disrupted nuclei, this fact may suggest that the DNA fragmentation that is induced by Al can be repaired and that not all TUNEL-positive nuclei become dis- rupted. Previous studies that used the comet assay showed that Al treatment resulted in an increase in DNA fragmentation thus indicating that Al directly affects DNA integrity in Arabi- dopsis roots [11]. No similar studies regarding the impact of Al on DNA integrity is known for barley.

Al has also been reported to delay cell divisions in root tips and inhibit DNA replication[32]. Recently, there has been a renewed interest in Al-induced alterations of the cell cycle, but most of these works are still focused on mitosis. Using flow cytometry analysis, we showed that after exposure to Al, the cell cycle profiles of the root tip cells differ from the profile of control roots. The frequency of cells in the G2/M phase increased after Al treatment and simulta- neously the frequency of the S-phase cells decreased. Similarly, Doncheva et al. [32] reported a decrease of S-phase cells in maize after short-term

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Al exposure and therefore an inhibition of root cell divisions. The decreasing of the frequency of S-phase in barley was similar as in Al- resistant maize variety, whereas S-phase was completely stopped in Al-sensitive variety.Although it is evident that Al causes cell cycle disturbances, many aspects are still unknown,e.g. the species-specific dependence and reversibility of these changes remain to be elucidated in future experiments.

Detection of DNA synthesis in proliferating cells is possible through the incorporation of labeled DNA precursors into DNA during the S phase of the cell cycle. Nowadays, the click reaction with 5-ethynyl-2'-deoxyuridine (EdU) is applied in studies related to DNA damage and cell cycle disturbances [43]. In this study, the visualization of nuclei with DNA synthesis using EdU permitted the analysis of the effect Al on the DNA replication in barley root tips. The results confirmed the effect of Al treatment on the frequency of S-phase cells. It can be assumed that the cells did not enter the S phase as a response to Al. At the same time, the S- phase cells entered the G2/M phase, and therefore an increase in the frequency of cells in these phases was observed. The effects of Al have been studied in detail in Arabidopsis roots [23, 44– 45]. To understand the Al impact on DNA damage and the cell cycle, a mutagenesis approach was used and resulted in the identification of Arabidopsis mutants with a hypersensitivity to Al. Using Arabidopsis mutants, it has been shown that Al causes the terminal differentiation of root tips and endoreduplication, together with a halting of the cell cycle progression in con- junction with a loss of the root-quiescent center [11,12].

The results of this study may help to understand the mechanism of Al action in barley cells.It is important to get know the processes that underlie Al toxicity under specific conditions including a species or cultivar sensitivity, medium composition, Al concentration and the duration of Al exposure.

'Sebastian' to Al contrasted with different species. The instrument that is answerable for the diminished cell division rate in roots after Al treatment might be associated with the immediate Al tie ing to the DNA phosphate spine [37,38]. The cytogenetic impacts of Al treatment in grain that was seen in the gave examination were perfect the adjustments in the root development boundaries, for example, decline in the all out root length, all out root zone and all out root volume.

Point by point investigations of the side effects of root development hindrance after Al treatment, which have not recently been accounted for grain, were conceivable utilizing a particular root scanner combined with the WinRHIZO programming. These examinations are anything but difficult to deal with and snappy and subsequently ace vide significant information to anticipate the cytogenetic impacts that are liable for root restraint, in this manner supplanting the tedious investigations in Al-advancing tests.

The effect of Al on DNA has just been proposed [38]. The impacts of Al3+ particles on DNA honesty, which are seen as micronuclei, have been shown in numerous species. Minet al. [39] detailed a noteworthy increment in the recurrence of micronuclei in Vicia faba root tip cells after Al treatment in the range 0.01–10 mM. Chromosome variations prompted by Al have likewise been accounted for in wheat [40] and rice [41]. Our outcomes show that Al is a powerless clastogenic specialist in Hordeum vulgare cultivar 'Sebastian' cells that are presented to the tried Al fixations in a scope of 20–40 μM. We likewise found that Al upset the morphology of cores, which has not recently been accounted for. This impact might be one of the indications of cell passing that is actuated by Al. The investigations of Pan et al. [13] depicted a few parts of star grammed cell passing (PCD) and recommended that Al can prompt this procedure in grain and other plant species. Al-actuated cell demise has been concentrated in six oat species including maize, wheat, triticale, rye, grain and oat [42]. DNA discontinuity, which was examined electropho-retically and showed PCD, was seen in rye, grain and oat roots, however not in maize and wheat. These outcomes recommend that wheat and maize are more open minded to Al than the other ana-lyzed species [42]. Information from our examination utilizing the TUNEL test affirmed that Al treatment actuated DNA fracture in the grain root tip cells and hence bolster this hypothesis about PCD. The recurrence of emphatically marked cores in the TUNEL test was altogether dif-ferent from the control simply after treatment with 40 μM Al. As TUNEL-positive cells happened more every now and again than the disturbed cores, this reality may recommend that the DNA fracture that is incited by Al can be fixed and that not all TUNEL-positive cores become dis-rupted. Past investigations that utilized the comet measure demonstrated that Al treatment brought about an expansion in DNA fracture in this way showing Al straightforwardly influences DNA uprightness in Arabi-dopsis roots [11]. No comparable examinations with respect to the effect of Al on DNA trustworthiness is known for grain.

Al has additionally been accounted for to defer cell divisions in root tips and restrain DNA replication[32]. As of late, there has been a reestablished enthusiasm for Al-incited adjustments of the cell cycle, however the vast majority of these works are as yet centered around mitosis. Utilizing stream cytometry investigation, we demonstrated that after presentation to Al, the cell cycle profiles of the root tip cells contrast from the profile of control roots. The recurrence of cells in the G2/M stage expanded after Al treatment and simulta-neously the recurrence of the S-stage cells diminished. Likewise, Doncheva et al. [32] detailed a reduction of S-stage cells in maize after transient Al introduction and subsequently a restraint of root cell divisions. The diminishing of the recurrence of S-stage in grain was comparable as in Al-safe maize assortment, though S-stage was totally halted in Al-delicate variety.Although it is obvious that Al causes cell cycle aggravations, numerous perspectives are still unknown,e.g. the species-explicit reliance and reversibility

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of these progressions stay to be explained in future examinations.

Identification of DNA amalgamation in multiplying cells is conceivable through the consolidation of named DNA forerunners into DNA during the S period of the phone cycle. These days, the snap response with 5-ethynyl-2'- deoxyuridine (EdU) is applied in examines identified with DNA harm and cell cycle unsettling influences [43]. In this examination, the representation of cores with DNA blend utilizing EdU allowed the investigation of the impact Al on the DNA replication in grain root tips. The outcomes affirmed the impact of Al treatment on the recurrence of S-stage cells. It very well may be accepted that the cells didn't enter the S stage as a reaction to Al. Simultaneously, the S-stage cells entered the G2/M stage, and consequently an expansion in the recurrence of cells in these stages was watched. The impacts of Al have been concentrated in detail in Arabidopsis roots [23, 44–45]. To comprehend the Al sway on DNA harm and the cell cycle, a mutagenesis approach was utilized and brought about the recognizable proof of Arabidopsis freaks with an excessive touchiness to Al. Utilizing Arabidopsis freaks, it has been demonstrated that Al causes the terminal separation of root tips and endoreduplication, along with an ending of the cell cycle movement in con-intersection with lost the root-calm focus [11,12].

The aftereffects of this examination may assist with understanding the component of Al activity in grain cells.It is essential to get know the procedures that underlie Al poisonousness under explicit conditions including an animal groups or cultivar affectability, medium piece, Al fixation and the span of Al introduction.