*Pak. J. Bot.*, 54(1): 125-130, 2022. DOI: http://dx.doi.org/10.30848/PJB2022-1(8)

# EXOGENOUSLY APPLIED ALUMINUM INDUCED GROWTH INHIBITION AND APOPTOSIS IN WHEAT SEEDLINGS

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**Abstract**

Aluminum (Al) toxicity significantly limits plant productivity, hinders plant growth, and reduces food production in most crops, including wheat. The present study explored the effects of $AlCl_3$ on wheat seedlings at the physiological and cytological levels. The results showed that $Al^{3+}$ stress affected chlorophyll synthesis in wheat seedling leaves. We found that chlorophyll content and $Al^{3+}$ concentration are negatively correlated in wheat seedlings. Root length, number of adventitious roots, and cell division index at the root tip showed a negative correlation with $Al^{3+}$ concentration. Besides, $Al^{3+}$ stress caused chromosomal aberrations in wheat seedlings, such as micronuclei and polynuclear formation, chromosomal breakage, the formation of a chromosomal bridge, and chromosomal loop. Exogenous application of excess Al could cause $H_2O_2$ accumulation in meristematic and elongation regions of the root, membrane oxidation, programmed cell death, and DNA damage. These results showed that excessive application of exogenous Al inhibited the normal growth of wheat and affected various physiological activities in wheat. Therefore, Al toxicity severely affects the growth and development of wheat seedlings.

**Key words:** Aluminum stress, Toxicity, Abnormal division.

## Introduction

Aluminum (Al) is the third most abundant metallic element in the earth's crust and a significant impediment for plant growth in acidic soils (Campos & Viccini, 2003; Xu *et al*., 2018), as it covers approximately 50% of the potentially arable land globally. Besides, the soil also undergoes acidification during formation (Kochian *et al*., 2015). For instance, acid rain would gradually reduce the soil pH, thus decline the quality of the soil. Besides, the excessive use of chemical fertilizers on cultivated land and discharge of wastewater from factories causes soil acidification (Matsumoto, 2000; Guo *et al*., 2010; Lu *et al*., 2011; Siecińska *et al*., 2019). As the acidity level of the soil increases, $Al^{3+}$ can damage plants severely.

Wheat (*Triticum aestivum* L.) is the most widely cultivated monocotyledonous plant in the world. As one of the main food crops, wheat is an essential nutrient source for humans. Besides drought, Al stress is the second-largest abiotic limiting factor in acid soils, which results in lower yields and crops of inferior quality (Kong *et al*., 2000; Shen & Yan, 2002; He *et al*., 2019). Since photosynthesis is sensitive to stress response, plant photosynthetic competence is a significant marker for evaluating plant resistance to stress. The photosynthetic function reflects in two aspects; chlorophyll content and chlorophyll fluorescence kinetics. The chlorophyll content is typically a reflection of plant stress resistance and indicates the photosynthetic capacity and plant growth (Stone *et al*., 1996; Cartelat *et al*., 2005). Meanwhile, the chlorophyll fluorescence kinetics of a leaf can quickly evaluate light energy absorption, transfer, consumption, and distribution during photosynthesis (Carcamo *et al*., 2019). Anthocyanins are secondary plant metabolites with numerous physiological and biochemical roles. Notably, the anthocyanin content in plants can reveal the correlation between stress and anthocyanin synthesis. Therefore, chlorophyll and anthocyanin contents are essential indicators of the physiological state of plants, and can thus directly reflect the growth state of plants (Garriga *et al*., 2014). Plants under Al stress portray several toxic symptoms, including slow growth, dwarfness, local yellowing of leaves, and decreased yield, among others.

Plant cells respond to exogenous stimuli in a variety of ways, such as through Programmed cell death (PCD). PCD is an active process of physiological cell death regulated by complex genetic mechanisms. PCD in plants and animals has some common morphological characteristics, such as cytoplasmic shrinkage, nuclear concentration, activation of specific proteases, DNA fragmentation, and the formation of apoptotic bodies (Pennell & Lamb, 1997; Solomon *et al*., 1999). When stressed, plants experience an outburst of reactive oxygen species (ROS) (Liu *et al*., 2018). Subsequently, the ROS activate the signal transduction pathway to induce cell death (Pan *et al*., 2001; Zhou *et al*., 2018), indicating that ROS are involved in cell signal transduction (Van *et al*., 2001), aerobic metabolism, PCD, and other processes (Bhattacharjee, 2005). Although it is inadvertently known that the root tip is the most stress-sensitive part of a plant, the mechanism by which Al inhibits wheat root growth remains unclear (Yamamoto *et al*., 2001; Barcelo & Poschenrieder, 2002; Kochian *et al*., 2004). Some studies have suggested that Al stress cauld affect the normal division of wheat root cells (Mohammed *et al*., 2013), and can also generate abnormalities in wheat cell chromosomes.

In this study, wheat seedlings were treated with aluminum chloride ($AlCl_3$) solutions of different concentrations to evaluate the physiological and

morphological effects of aluminum stress on wheat at the cellular and molecular levels. The results of the present study provide essential data for studying the response of wheat seedlings to aluminum stress, which is of great significance for wheat production and grain quality improvement.

## Materials and Methods

**Plant materials and growth conditions:** Screened wheat seeds with full and equal granules were provided by the wheat research institute of Shanxi Academy of Agricultural Sciences. The seeds were germinated in a biochemical incubator with constant temperature and illumination at 28°C, a light/dark cycle of 14 h/10 h, and a humidity of about 70%. The treatment group was irrigated with 10 mL $ddH_2O$ on the first day and then treated with an $Al^{3+}$ solution of different concentrations, daily for five days. The concentration gradients of the $Al^{3+}$ solution were 0, 5, and 10 mmol $L^{-1}$.

**Effects of $AlCl_3$ on fresh/dry weight of wheat seedling leaves:** The wheat seedling leaves were washed, dried, and weighed fresh using an electronic balance. Then, the weighed fresh leaves were wrapped tightly in a tin foil, placed in a drying box maintained at 80°C for 24 h, and weighed to obtain the dry weight. The fresh/dry weight of wheat seedling leaves in each group was measured, and the average value was calculated.

**Effects of $AlCl_3$ on the chlorophyll and anthocyanin contents of wheat seedling leaves:** For the determination of chlorophyll content (Gitelson *et al*., 2003), 0.5 g wheat leaves from each treatment group were weighed, cut, and an 8 mL mixture of acetone and methanol (1:1) was added. The leaves were dark-incubated at room temperature until completely decolorized. The optical density (OD) of the supernatant was evaluated at wavelengths of 663 nm and 645 nm. For the determination of anthocyanin content method of (Hamilton-Amachree & Etasi, 2019) was followed, 0.5 g of wheat seedling leaves from each treatment group were weighed, cut, and transferred to a mortar. Subsequently, 2 mL of hydrochloric acid (pH=1.28) was added, and the mixture was quickly ground. The crude lysate was transferred to a 5 mL EP tube and placed at room temperature for 2 h. The OD of the supernatant was evaluated at a wavelength of 525 nm.

**Morphological changes of wheat seedling roots:** The root lengths of ten wheat seedlings in each of the three experimental groups were measured with a ruler, and the mean values were calculated.

**Carbol Fuchsin Solution staining of wheat seedling root tips:** Carbol Fuchsin Solution staining was done to evaluate cell division at the root tips of wheat seedlings. The root tips of wheat seedlings were fixed in solution (methanol: acetic acid = 3:1) for 4 h, soaked in 95% ethanol for 40 min, then transferred to 70% alcohol. After that, the fixed plant tissues were rinsed with distilled water 2-3 times, hydrolyzed in 1 mol $L^{-1}$ HCl at 60°C for 12 min, and then washed with distilled water. The tissue was placed at the center of the slide, the dye solution added and left standing for 15 min, and the slide was cover-slipped. The morphology of chromosomes was observed under the microscope (Olympus IX 71), and the mitotic index, micronucleus percentage, and chromosome aberration rate were calculated.

**DAPI staining of wheat seedling root tips:** The root tips of wheat seedlings from different treatment groups were placed in different 1.5 mL EP tubes. Subsequently, 1 mL of $ddH_2O$ and 10 uL of DAPI (4', 6-diamidino-2-phenylindole) solution (100 ng $mL^{-1}$) were added to the EP tube and dark-incubated for 15 min. Then, the roots were ashed 3-5 times with $ddH_2O$, and the cells in root tips were observed by pressing.

**Detection and fluorescence localization of ROS in wheat seedling root tips:** For fluorescence localization, the root tips of wheat seedlings were put in an $H_2$DCF-DA solution (2',7'-Dichlorodihydrofluorescein diacetate) and dark-treated for 15 min. The root tips of wheat seedlings from different treatment groups were placed in different 2 mL EP tubes. After washing twice with $ddH_2O$, the fluorescence intensity of the root tip cells was observed under a fluorescent microscope (Olympus IX 71).

**DNA extraction and gel electrophoresis of wheat seedlings:** Genomic DNA was extracted using the CTAB method with modifications (Jenkins *et al*., 2012). Briefly, liquid nitrogen was added to 1 g of plant tissue, ground thoroughly and then 1 mL of CTAB solution preheated at 60-65°C was added. The mixture was incubated in a water bath at 65°C for 20 min, and then an ice bath for 10 min. Then, 1 mL of freshly prepared chloroform/ isoamyl alcohol (24:1) was added. Centrifuged at 12000rpm for 2 min, pre-cooled isopropanol was added to the supernatant to precipitate the DNA, and the centrifugation step was repeated. The supernatant was discarded, and DNA was dissolved in 30-50 uL of $ddH_2O$. Finally, the DNA was resolved in a 1% agarose gel for the detection quality of DNA.

## Data statistics

All experiments were repeated at least three times. The SPSS software version 25.0 was used for relevant data processing. Differences between the mean values of each attribute were evaluated by univariate analysis of variance.

## Results and Discussion

### Wheat seedlings under aluminum stress

**Phenotypic abnormalities of wheat seedlings under aluminum stress:** The plant height, fresh weight, and dry weight are essential indicators of the growth status of wheat. It was found that the plant height of wheat seedlings decreased with an increase in $Al^{3+}$ concentration (Fig. 1a), $p<0.05$. While the fresh seedling weight was decreased significantly with an increase in $Al^{3+}$ concentration, the dry weight of each treatment group showed little difference (Fig. 1b). With increase in $Al^{3+}$ concentration, the root

length and adventive root number of wheat seedlings decreased gradually (Fig. 1c-1d). The average root length of the control group was 4.575 cm, while that of the 5 and 10 mmol $L^{-1}$ $Al^{3+}$ treatment groups was 0.857 cm and 0.500 cm, respectively. The average number of adventive roots in the control group was five, while that of the 5 and 10 mmol $L^{-1}$ $Al^{3+}$ treatment groups were 4 and 3, respectively. We found that Al toxicity could significantly limit the length and number of roots in wheat seedlings, and also affected the water and nutrient uptake of the seedlings. Thus, our results showed that Al had a toxic effect on the root tip cells of wheat seedlings. In conclusion, the phenotype of wheat under Al stress was abnormal.

**Changes in the chlorophyll and anthocyanin contents of wheat seedling leaves under aluminum stress:** Chlorophyll could reflect the photosynthetic intensity of plants. Relative to the control group, the chlorophyll content of the 5 mmol $L^{-1}$ and 10 mmol $L^{-1}$ treatment groups was reduced by 51.6% and 73.3%, respectively. Compared with the 5 mmol $L^{-1}$ treatment group, the 10 mmol $L^{-1}$ treatment group showed a relatively lower decline in chlorophyll content (Fig. 1e). A particular concentration of $Al^{3+}$ could cause a sharp decline in the chlorophyll content of plant leaves. Beyond a specific range, however, the decrease in chlorophyll content would be alleviated, which could be related to the plant's stress resistance. This finding indicates that plants activate their anti-stress mechanisms to resist stress and minimize cell damage. Anthocyanin is a natural plant pigment that plays multiple functions. For instance, anthocyanins could aid stress resistance in plants. Compared with the control group, the anthocyanin contents of both the 5 mmol $L^{-1}$ and 10 mmol $L^{-1}$ treatment groups did not change significantly, $P$=0.285>0.05 (Fig. 1f). Therefore, anthocyanin biosynthesis was not considerably affected by $Al^{3+}$ treatment, which could be because anthocyanins are closely related to plant stress resistance.

**Abnormal mitosis in root tip cells of wheat seedlings:** The root tip cells of wheat seedlings showed abnormal mitotic activity under Al stress, as evidenced by the production of multi-nucleus and micro-nucleus in the cell. The micronucleus production rate of root tip cells increased significantly with increase in $Al^{3+}$ concentration, $p$<0.05 (Table 1, Fig. 2a-2f). Under Al stress, the nuclear morphology of the root tip cells of wheat seedlings changed, and amitosis was observed (Fig. 2g-2i). Besides, the root tip cells of wheat seedlings developed chromosome aberrations under Al stress (Table 1, Fig. 2j-2r). Al toxicity leads to the appearance of micronucleus, which signifies cellular abnormality. Besides, Al toxicity also causes chromosomal aberrations. Chromosomes in the nucleus contain genetic information, and chromosomal mutations could, therefore, affect the normal growth of plants.

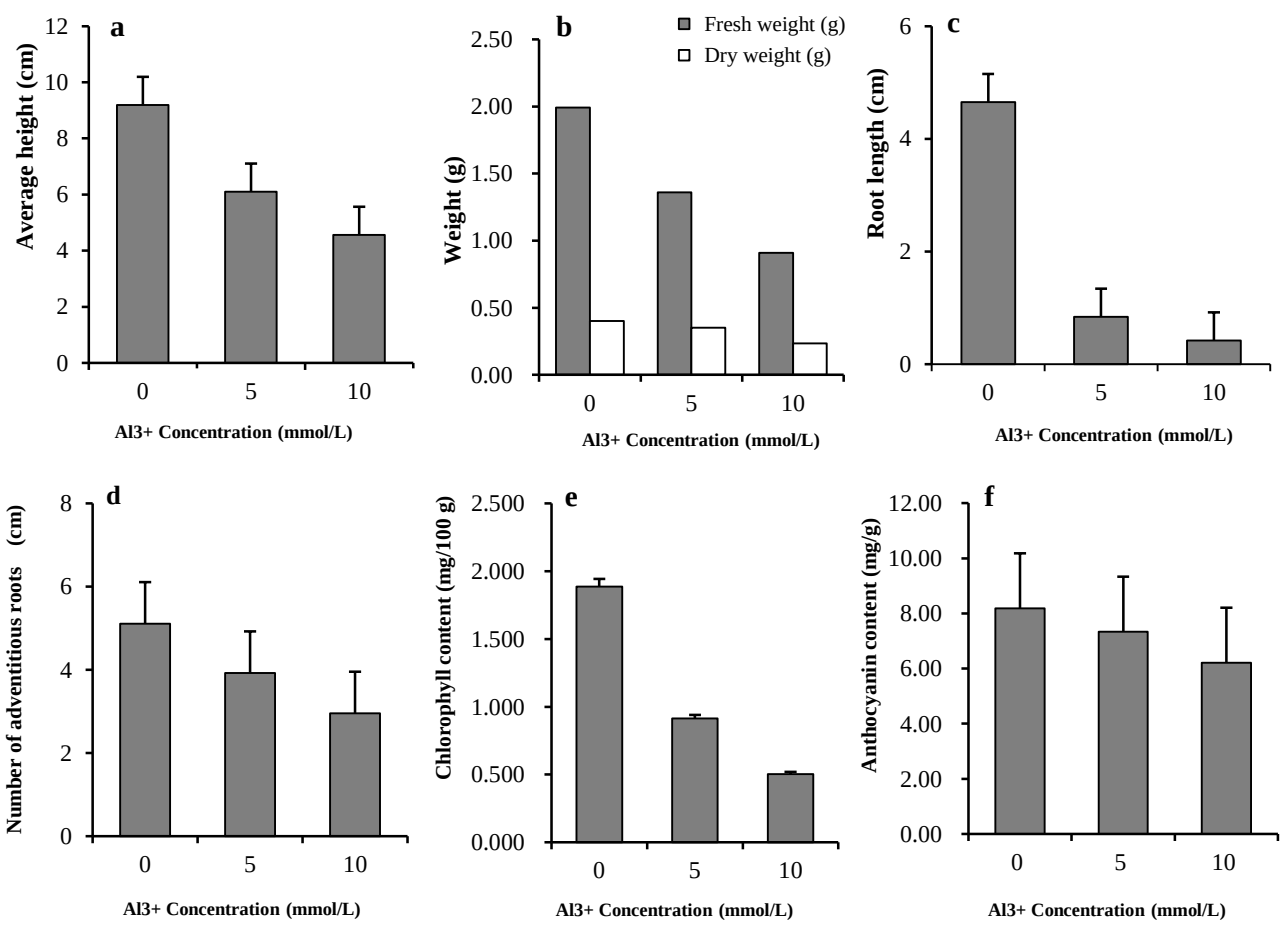


Fig. 1. Changes in wheat seedlings under aluminum stress.
Plant height (a); Fresh/dry weight (b); Root length (c); Adventitious root number (d); Chlorophyll content (e); Anthocyanin content (f)

**Table 1. Effect of aluminum stress on micronucleus formation rate and chromosomal aberration rate of root tip cells.**

| Concentration (mmol $L^{-1}$) | Micronucleus rate (%) | Aberration rate (%) |
|---|---|---|
| 0 | 6.20 ± 1.41a | 1 ± 1.41a |
| 5 | 18.50 ± 3.54b | 14.00 ± 1.41b |
| 10 | 30.00 ± 1.41c | 18.50 ± 0.71c |

Note: Different letters in the data indicate significant differences between treatment groups ($p$<0.05)

Plants can respond to stress from all forms of adversity. By examining the mechanism by which the root tip cells of aluminum-tolerant wheat resist aluminum toxicity, researchers found that different wheat varieties respond differently under aluminum stress (Wu *et al*., 2018). Similar to our results, one study reported that cadmium toxicity induced chromosomal aberrations and micronucleus formation in Triticale. These findings suggested that cadmium was also toxic to root tip cells. Certain factors can cause the formation of micronuclei. For instance, Yi *et al*., (2010) showed that Al toxicity increases the micronucleus formation rate in the root tip cells of broad bean (*Vicia faba* L.) and causes cellular distortion. The findings of the above studies are consistent with the results of this experiment. The research found two, three, and six nuclei in the root tip cells of Alnus (*Alnus cremastogyne* Burk.). Other scholars suggested that amitosis can cause multiple nuclear phenomena (Marciniak, 1991). In this study, Al stress also induced the double-and triple-nucleus events in wheat root tip cells.

**ROS accumulation in root tip cells of wheat seedlings:** The ROS content in plants could intuitively reflect the degree of cellular oxidation. The fluorescence intensity of the cells could reveal the $H_2O_2$ content of wheat seedling root tip cells after $H_2$DCF-DA staining. Under Al stress, the fluorescence intensity of the treatment groups was more intense (Fig. 3b-3c). This result indicates that $H_2O_2$ accumulates in the cells of wheat seedlings under Al stress. $H_2O_2$ accumulation could cause membrane peroxidation in cells and damage the integrity of the cell membrane. Plant stress damages the components of cell walls and membranes. It has been shown that Al stress intensifies the permeability of plant cell membrane, indicating that Al stress can cause peroxidation of plant root tips and harm the plasma membrane. If ROS is not eliminated promptly, it could damage cellular DNA and cause cell death in severe cases.

**DAPI staining in root tip cells of wheat seedlings:** DAPI staining can detect apoptosis. In this study, many apoptotic bodies were found in the elongation region of root tip cells in the 10 mmol $L^{-1}$ treatment group (Fig. 3d-3e). Thus, we showed that a high concentration of $Al^{3+}$ could trigger apoptosis in cells. Excessive $Al^{3+}$ affects the normal growth of plant roots and severely damages the structure and function of root tips. In the present study, DAPI staining successfully revealed morphological changes of the nucleus in the root tip cells of wheat seedlings. The number of heterotypic cells increased with an increase in $Al^{3+}$ concentration, and a typical "meniscus" nucleus was observed in the treatment groups (Fig. 3g-3i). The above results verify that Al stress could hinder the normal growth of plant cells and, thus, affect various life activities of plants.

**DNA damage in wheat seedlings under aluminum stress:** The DNA of wheat seedlings exposed to Al stress showed evident DNA ladder-like bands and a clear trailing phenomenon. The brightness of the tailing band of DNA increased with increase in $Al^{3+}$ concentration, indicating that Al stress sheared the DNA of wheat seedling cells (Fig. 3j). As the concentration of $Al^{3+}$ increased, more small fragments of DNA were broken, thus increasing the band's brightness. This observation indicates that high levels of $Al^{3+}$ could enhance necrosis in plant cells. In the control group, there was also a small amount of some minimal DNA shearing was also evidenced, which could have been caused by cultivating wheat seedlings in solution for a long time. Thus, the seedling roots became hypoxic, leading to the formation of aerenchyma tissues in the roots. The apoptosis which occurred during aerenchyma formation could have also resulted in partial shearing of the DNA.

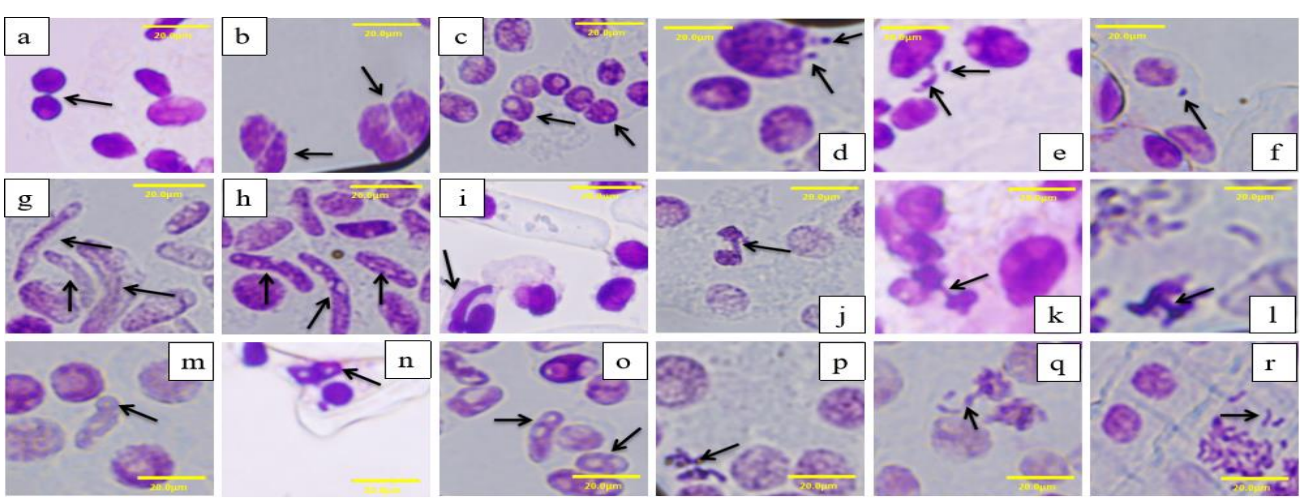


Fig. 2. Multinuclear and micronuclei in wheat seedling cells under aluminum stress.
Multinucleated and micronucleated cells (a-f); The nuclear morphology of the root tip cells of wheat seedlings were changed, and amitosis occurred in the cells under Al stress (g-i); Late-stage bridge appeared in the root tip cells of wheat seedlings under Al stress (j-l); Chromosomal rings appeared in the root tip cells of wheat seedlings under Al stress (m-o); Chromosomal enrichment, chromosomal breakage, and loss in root tip cells of root tips of wheat seedlings under Al stress (p-r).

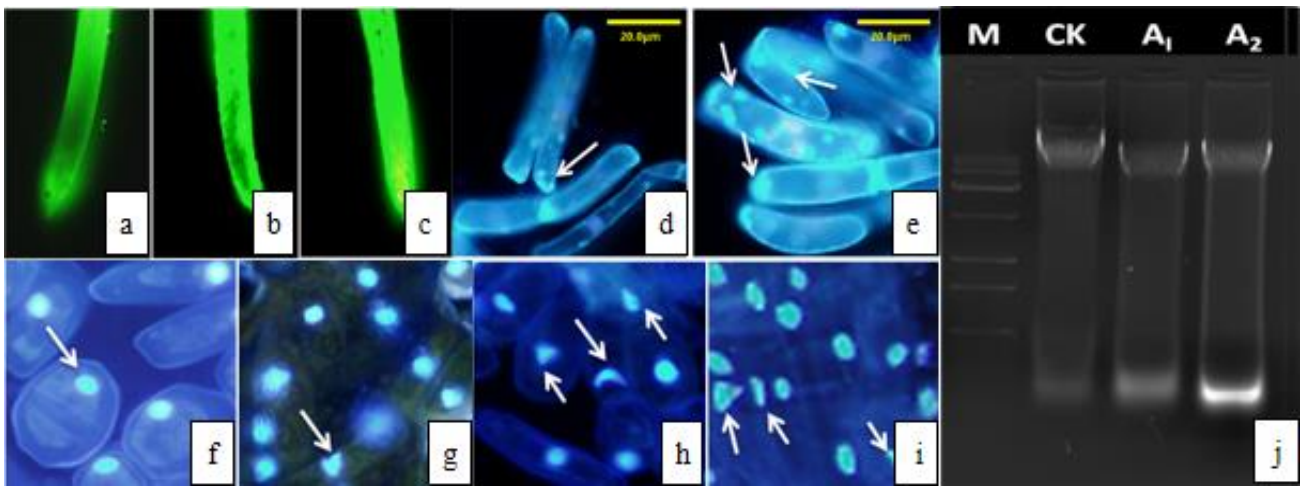


Fig. 3. Accumulation of $H_2O_2$ in root tip cells of wheat seedlings under aluminum stress
Accumulation of $H_2O_2$ in root tip cells of wheat seedlings under Al stress (a-c); Apoptosis in root tip cells of wheat seedlings under Al stress (d-e); Differences in the nuclear shape of the root tip of wheat seedlings under Al stress (f-i); DNA damage of wheat seedlings under Al stress (j).

## Conclusions

Al stress inhibited the synthesis of chlorophyll in wheat and the growth of seedling root was inhibited, which led to the slow down of cell division in root region. At the same time, Al toxicity can also reduce the cell division index and increase the chromosome aberration in the nucleus. Al stress increases the ROS content of root tip cells and reduces the integrity of plant cell membrane, thus affecting the normal growth of plants. Al stress has some genotoxicity. Therefore, it is necessary to study how to reduce the toxicity of aluminum and prevent aluminum pollution.

## Acknowledgments

This work was supported by the Natural Science Foundation of China under Grant No. 31900251.

### References

Barcelo, J. and C. Poschenrieder. 2002. Fast root growth responses, root exudates, and internal detoxification as clues to the mechanisms of aluminium toxicity and resistance: a review. *Environ. Exp. Bot.*, 48(1): 75-92.

Bhattacharjee, S. 2005. Reactive oxygen species and oxidative burst: roles in stress, senescence and signal transducation in plants. *Curr. Sci. India*, 89(7): 1113-1121.

Breusegem, F.V., E. Vranova, J.F. Dat and D. Inze. 2001. The role of active oxygen species in plant signal transduction. *Plant Sci.*, 161(3): 405-414.

Campos, J.M.S.D. and L.F. Viccini. 2003. Cytotoxicity of aluminum on meristematic cells of *Zea mays* and *Allium cepa*. *Caryologia*, 56(1): 65-73.

Carcamo, M.P., M. Reyesdiaz, Z. Rengel, M. Alberdi, R.P. Omena-Garcia, A. Nunes-Nesi and C. Inostroza-Blancheteau. 2019. Aluminum stress differentially affects physiological performance and metabolic compounds in cultivars of highbush blueberry. *Sci. Rep.*, 9(1): 1-13.

Cartelat, A., Z.G. Cerovic, Y. Goulas, S. Meyer, C. Lelarge, J.L. Prioul and I. Moya. 2005. Optically assessed contents of leaf polyphenolics and chlorophyll as indicators of nitrogen deficiency in wheat (*Triticum aestivum* L.). *Field Crop. Res.*, 91(1): 35-49.

Garriga, M., J.B. Retamales, S. Romero-Bravo, P.D. Caligari and G.A. Lobos. 2014. Chlorophyll, anthocyanin, and gas exchange changes assessed by spectroradiometry in Fragaria chiloensis under salt stress. *J. Integr. Plant Biol.*, 56(5): 505-515.

Gitelson, A.A., Y. Gritz † and M.N. Merzlyak. 2003. Relationships between leaf chlorophyll content and spectral reflectance and algorithms for non-destructive chlorophyll assessment in higher plant leaves. *J. Plant Physiol.*, 160(3): 271-282.

Guo, J.H., X.J. Liu, Y. Zhang, J.L. Shen, W.X. Han, W.F. Zhang and F.S. Zhang. 2010. Significant acidification in major Chinese croplands. *Science*, 327(5968): 1008-1010.

Hamilton-Amachree, A. and N.J. Etasi. 2019. Spectrophotometric Determination of Total Anthocyanin Content (TAC) in Costus afer Flower Extract and its Application as pH Indicator in Acid/Base Titrations. *J. Chemical Soc. Nigeria*, 44(2): 341-347.

He, H., Y. Li and L.F. He. 2019. Aluminum toxicity and tolerance in Solanaceae plants. *S. Afr. J. Bot.*, 123: 23-29.

Jenkins, G.R., J.T. Helber and L.D. Freese. 2012. Concordance study: methods of quantifying corn and soybean genomic DNA intended for real-time polymerase chain reaction applications. *J. Agricult. & Food Chem.*, 60(34): 8323-8332.

Kochian, L.V., M.A. Piñeros, J. Liu and J.V. Magalhaes. 2015. Plant adaptation to acid soils: the molecular basis for crop aluminum resistance. *Annu. Rev. Plant Biol.*, 66: 571-598.

Kochian, L.V., O.A. Hoekenga and M.A. Hoekenga. 2004. How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorous efficiency. *Annu. Rev. Plant Biol.*, 55(1): 459-493.

Kong, F., W. Sang, X. Jiang and L. Wang. 2000. Aluminum toxicity and tolerance in plants. *Acta Ecologica Sinica*, 20(5): 855-862.

Liu, W., F. Xu, T. Lv, W. Zhou, Y. Chen, C. Jin and X. Lin. 2018. Spatial responses of antioxidative system to aluminum stress in roots of wheat (*Triticum aestivum* L.) plants. *Sci. Total. Environ.*, 627: 462-469.

Lu, W., Y. Ma and C. Lin. 2011. Status of aluminium in environmental compartments contaminated by acidic mine water. *J. Hazard. Mater.*, 189(3): 700-709.

Marciniak, K. 1991. Cytophotometric analysis of amitosis in nuclear endosperm of *Vicia faba*. *Folia Histochem. Cyto.*, 29(2): 67-69.

Matsumoto, H. 2000. Cell biology of aluminum toxicity and tolerance in higher plants. *Int. Rev. Cytol.,* 1-46.

Mohammed, Y.S.A., A.E. Eltayeb and H. Tsujimoto. 2013. Enhancement of aluminum tolerance in wheat by addition of chromosomes from the wild relative *Leymus racemosus*. *Breeding Sci.,* 63(4): 407-416.

Pan, J.W., M.Y. Zhu and H. Chen. 2001. Aluminum-induced cell death in root-tip cells of barley. *Environ. Exp. Bot.,* 46(1): 71-79.

Pennell, R.I. and C. Lamb. 1997. Programmed cell death in plants. *The Plant Cell,* 9(7): 1157-1168.

Shen, H. and X. Yan. 2002. Exudation and accumulation of organic acids in the roots of common bean (*Phaseolus vulgaris* L.) in response to low phosphorus and aluminum toxicity stress. *Acta Ecologica Sinica,* 22(3): 387-394.

Siecińska, J., D. Wiącek, B. Przysucha and A. Nosalewicz. 2019. Drought in acid soil increases aluminum toxicity especially of the Al-sensitive wheat. *Environ. Exp. Bot.,* 165: 185-195.

Solomon, M., B. Belenghi, M. Delledonne, E. Menachem and A. Levine. 1999. The involvement of cysteine proteases and protease inhibitor genes in the regulation of programmed cell death in plants. *The Plant Cell,* 11(3): 431-443.

Stone, M.L., J.B. Solie, W.R. Raun, R.W. Whitney, S.L. Taylor and J.D. Ringer. 1996. Use of spectral radiance for correcting in-season fertilizer nitrogen deficiencies in winter wheat. *T. Asabe.,* 39(5): 1623-1631.

Wu, L., J. Yu, Q. Shen, L. Huang, D. Wu and G. Zhang. 2018. Identification of microRNAs in response to aluminum stress in the roots of Tibetan wild barley and cultivated barley. *BMC Genomics,* 19(1): 560-.

Xu, L., C. Liu, B. Cui, N. Wang, Z. Zhao, L. Zhou, K. Huang, J. Ding, H. Du, W. Jiang and S. Zhang. 2018. Transcriptomic responses to aluminum (Al) stress in maize. *J. Integr. Agr.,* 17(9): 1946-1958.

Yamamoto, Y., Y. Kobayashi and H. Matsumoto. 2001. Lipid peroxidation is an early symptom triggered by aluminum, but not the primary cause of elongation inhibition in pea roots. *J. Plant Physiol.,* 125(1): 199-208.

Yi, M., H. Yi, H. Li and L. Wu. 2010. Aluminum induces chromosome aberrations, micronuclei, and cell cycle dysfunction in root cells of Vicia faba. *Environ. Toxicol.,* 25(2): 124-129.

Zhou, Y., Z. Yang, Y. Xu, H. Sun, Z. Sun, B. Lin, W. Sun and J. You. 2018. Soybean NADP-malic enzyme functions in malate and citrate metabolism and contributes to their efflux under Al stress. *Front. Plant Sci.,* 8: 2246.

(Received for publication 26 April 2020)