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Broad Hormonal Responses Induced by Aluminum in Roots of Dwarf Transgenics of *Solanum lycopersicum* L. cv "Micro-Tom"

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Abstract

The spatial pattern distribution of plant hormones in response to aluminum (Al) toxicity in roots remains to be shown. This study was performed to assess the root hormonal accumulation and gene expression in response to Al toxicity in five transgenic miniature dwarf tomatoes cv. Micro-Tom (MT). MT and MT transgenics to acid indole acetic, cytokinin, gibberellin, abscisic acid and ethylene were cultivated in nutrient solutions containing different Al concentrations. Root growth elongation was measured and cellular damage was visualized by staining Evans's blue. The GUS reporter gene staining technique was used to visualize hormonal changes in MT apex root tissues. Data indicated that the MT is sensitive to Al that induced significant growth inhibition and cellular damage. Al concentration of 27 μM was significantly toxic, inducing root apex darkening and inhibition of root development. The qualitative evaluation of GUS reporter gene expression showed intense crosstalk among all hormones studied, underscoring the complexity of signaling induced by Al in apex roots. Results point out to a major understanding of the hormonal signaling in response to Al toxicity, which may induce a change of root growth and architecture with growth inhibition and cell constraints modulated by all different hormones evaluated.

Keywords

Gene Reporter, Metal, Plant Hormones, Root Staining

1. Introduction

Extensive research has demonstrated that aluminum (Al) alters several physiological processes and compromise plant growth and productivity [1], distur-

bance of the cytosolic homeostasis of calcium (Ca) and alterations of cytoskeleton dynamics [2]. However, the main mechanisms adjacent to Al toxicity in plants are still widely unknown. Despite Al toxicity to most plant species, some can tolerate high concentrations of the metal, especially some grasses [3]. Several mechanisms of tolerance to Al have been proposed and likely involve an active site for phytohormone action in the root apex [4], changes in physiological and biochemical processes [5], and active transport of metabolites [6]. Phytohormone-mediated root growth inhibition in response to Al stress occurs through a crosstalk between ethylene (ET), cytokinins (CKs), salycilic acid (SA), jasmonic acid (JA) and abscisic acid (ABA) [7] [8] [9]. Interactions between different phytohormone pathways are essential in coordinating tissue outgrowth in response to abiotic stress. However, the spatial pattern distribution of these hormones in response to Al stress in roots remains to be shown. *Solanum lycopersicum* cv. Micro-Tom (MT) has been widely used as a research model, and several transgenic and mutant genotypes are available. Such genotypes allow a detailed study of hormonal responses and can contribute to a better understanding of Al tolerance and, conversely, the sensitivity response. Particularly, MT demonstrates practical advantages, because it can be cultivated under fluorescent light and has a short life span ranging from 70 to 90 days [10]. To address how Al-induced inhibition of root growth can be achieved by alteration of hormone distribution in root tips, the objective of this study was to evaluate if the different Al concentrations would inhibit distinctively MT root growth, and if concentration changes in the main hormone groups are related to the response to Al toxicity in roots of MT and different MT hormonal transgenics.

2. Methodology

2.1. Plant Material

Tomato (*Solanum lycopersicum* L.) cv. Micro-Tom plants were used for all tests performed. MT was used as control and compared to the transgenic genotypes MT-*pDR5*::GUS; MT-*pARR5*::GUS; MT-*pGA20x*::GUS; MT-*pRD29B*::GUS and MT-*pEBS*::GUS (Table 1). Seeds were disinfected using 5% sodium hypochlorite (HClO) plus 2 drops of detergent and mixed for 1 min and after 10 min drained

Table 1. Tomato transgenic plants (*Solanum lycopersicum* cv. Micro-Tom) (MT), carriers of the fused GUS reporter gene, to promote the region induced by the five hormones being studied.

Genotype	Description	Reference
MT-pDR5::GUS	Fused GUS to promote auxin induction	[11]
MT-pARR5::GUS	Fused GUS to promote cytokinin induction	[12]
MT-pGA2Ox::GUS	Fused GUS to promote gibberellin induction	[13]
MT-pRD298B::GUS	Fused GUS to promote abscisic acid induction	[14]
MT-pEBS::GUS	Fused GUS to promote ethylene induction	[15]

using a sieve and abundantly washed with distilled water. This experiment aimed at finding the Al concentration which inhibits approximately 50% of root growth. The results were used to determine concentrations for subsequent experiments with MT.

2.2. Nutrient Solution with Aluminum Treatments

The nutrient solution used for all tests was that described by [16] at 10% ionic strength. All Al-containing solutions were prepared with aluminum chloride ($AlCl_3$) with added Homopipes buffer [17] and 80 seeds were used per treatment, with 40 seeds per replicate. Five treatments were used with increasing Al concentrations: control without Al and with 5, 10, 20 and 40 μM of Al. The seeds were placed in 250 mL Erlenmeyers containing 50 mL of nutrient solution and placed under constant shaking and 12 h of light. Root images were obtained by scanning in an Epson Perfection V800 Scanner and ImageJ software [18] on the 3rd and 5th days of root growth.

2.3. Root Growth, Cell Viability and GUS Expression

For cell viability assays, 15 seeds were used per treatment, as follows: control without Al and treatments with 3, 9, 18 and 27 μM Al. Seeds were placed in trays and kept in the same conditions described for 7 days. After this period, root cell viability was evaluated by analyzing Evans' blue absorption by damaged cells. Roots were submerged in 5 mL 0.25% (w/v) Evans' blue for 10 min, washed in distilled water and taken to a Leica ICC50 HP microscope coupled to a digital camera and a Leica Measure module, as described in [19]. To quantify Evans' blue absorption, 0.5 cm long root tips were sectioned from 15 roots from each treatment. Roots from each treatment were placed in 3 flacons containing 300 μM dimethylsulfoxide (DMSO) for 2 h, totaling 5 root tips per flacon. After this period, the DMSO solution was removed from the flacons and analyzed in a spectrophotometer at 600 μm wavelength. β-glucuronidase (GUS) histochemical analysis was conducted in MT and MT transgenic plant roots. Twenty seeds from each genotype were used, divided in 10 seeds for the control and the remaining 10 for the 18 μM Al treatment. 48 h after germination, seeds were removed from treatments and analyzed. Five seeds from each treatment were placed in 1.5 mL tubes containing 300 μM GUS solution, 100 mM phosphate buffer at pH 7.0; 10 mM EDTA at pH 8.0; 0.5 mM potassium ferricyanide, 0.05% Triton X-100 and 10 mg·mL^{-1} X-Gluc (5-bromo-4-chloro-3-indolyl-beta-D-glucuronic acid, cyclohexylammonium salt) and 500 μM DMSO for 5 min. Roots were infiltrated in a vacuum 5 times, for 5 min each. Afterwards, they were incubated in darkness at 37˚C during 12 h, washed in distilled water and kept in 70% ethanol for 15 min. Then, they were once again washed in distilled water and immersed in 0.24 N HCl with 20% methanol, in a heating pad, at 57˚C, for 15 min. Subsequently, the solution was substituted for 7% NaOH in 60% ethanol for 15 min at room temperature. Samples were washed in distilled water and placed in 40%

ethanol for rehydration for 5 min, and then placed in 70% ethanol. Roots were finally taken to a microscope for image acquisition. The whole-root images were obtained using Leica Application Suite – LAS software and data was obtained by observing the intensity of the blue staining in cells and tissues resulting from GUS activity. Parametric analyses were tested using the D'Agostino and Pearson omnibus tests. Significant differences in evaluated parameters were determined by one-way ANOVA, and when between treatments, using the multiple-scale HSD Tukey test. Correlation analysis was conducted using the Pearson correlation coefficient.

3. Results and Discussion

3.1. Aluminum and Root Growth

After 3 days of Al exposure, the mean average root growth of control plants was significantly greater than other treatments (**Figure 1**). There was no statistical difference between three of the Al-treated groups, 5, 10 and 20 µM. However, seedlings subjected to 40 µM treatment differed significantly from all others, having the smallest mean root growth and this implies that Al inhibits root growth in a dose-dependent way (**Figure 1**). After these results, the Al concentration in subsequent experiments was updated to 0, 3, 9, 18 and 27 µM Al.

3.2. Root Cell Viability

MT roots subjected to increasing Al concentrations exhibited increasing losses of cell viability. Evans' blue uptake was significantly greater under increased Al concentration, being clearly enhanced under 27 µM of Al (**Figures 2(a)-(e)**). These results indicate cell damage caused by Al toxicity, especially in the root elongation zone and corroborated by other studies in pea [19] [20]. Evans blue staining results underscore the data obtained for root growth, which was similar after 3 days in all treatments except in the seedlings subjected to the highest amount of Al (40 µM) (**Figure 1**). Similarly, absorbance was significantly higher in plants treated with 27 µM Al (**Figure 2(f)**, **Figure 2(g)**). It was observed that

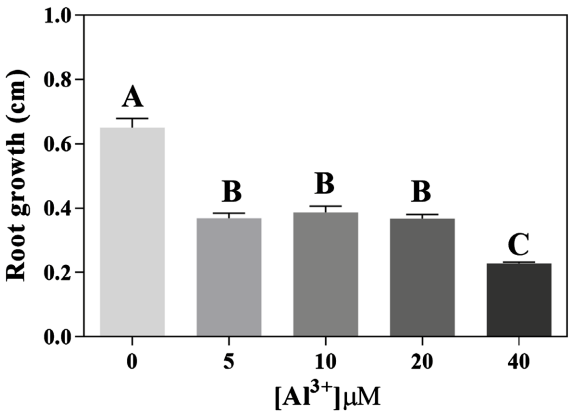

Figure 1. Root growth of MT plants subjected to increased Al concentrations. Bars represent means of 3 days of root growth with standard error. N = 80.

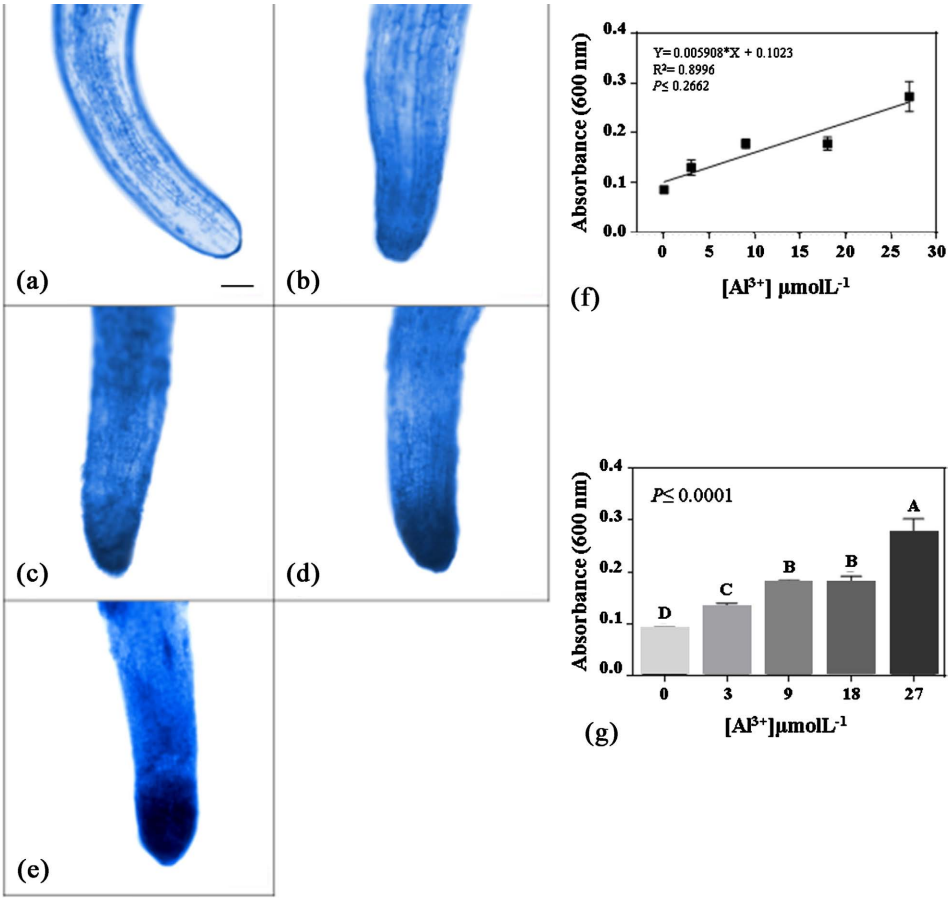

Figure 2. Cell viability expressed as Evans' blue staining intensity in root tips of MT after 7 days of treatment (a)-(e) with increasing Al concentrations (0, 3, 9, 18 and 27 µM). Bars represent 500 µm. (f) Linear regression correlation between Al treatments and Evans' blue absorption in root tissue. Bars represent standard deviation. Means: [0] = 0.0869; [3 µM Al] = 0.1310; [9 µM Al] = 0.1787; [18 µM Al] = 0.1786; [27 µM Al] = 0.2728.

the root tip began to exhibit tissue necrosis in seedlings subjected to 27 µM Al, demonstrating severe cell damage. High Al concentrations can block water and nutrient uptake mechanisms and can ultimately lead to cytological damage [20] and these effects can, consequently, cause root growth inhibition, which can be explained by elongation and cell division issues [21].

3.3. Root Histochemical Essay by GUS

MT plants were used as the negative control for GUS histochemical assays (**Figure 3(a)**, **Figure 3(b)**). Transgenic plants expressing DR5::GUS, which contain a synthetic promoter responsive to AIA [22], showed restricted expression in quiescent center cells of the root tip in the absence of Al (**Figure 3(c)**). GUS activity increased after Al exposure, encompassing the entire apical meristem region (**Figure 3(d)**). Root growth is also intimately related to the synthesis, transport, and distribution of auxin [7]. Aluminum may interact with the polar transport of AIA and therefore alter its accumulation in roots [23]. In turn, these changes in AIA distribution contribute to growth inhibition. Indeed, our results

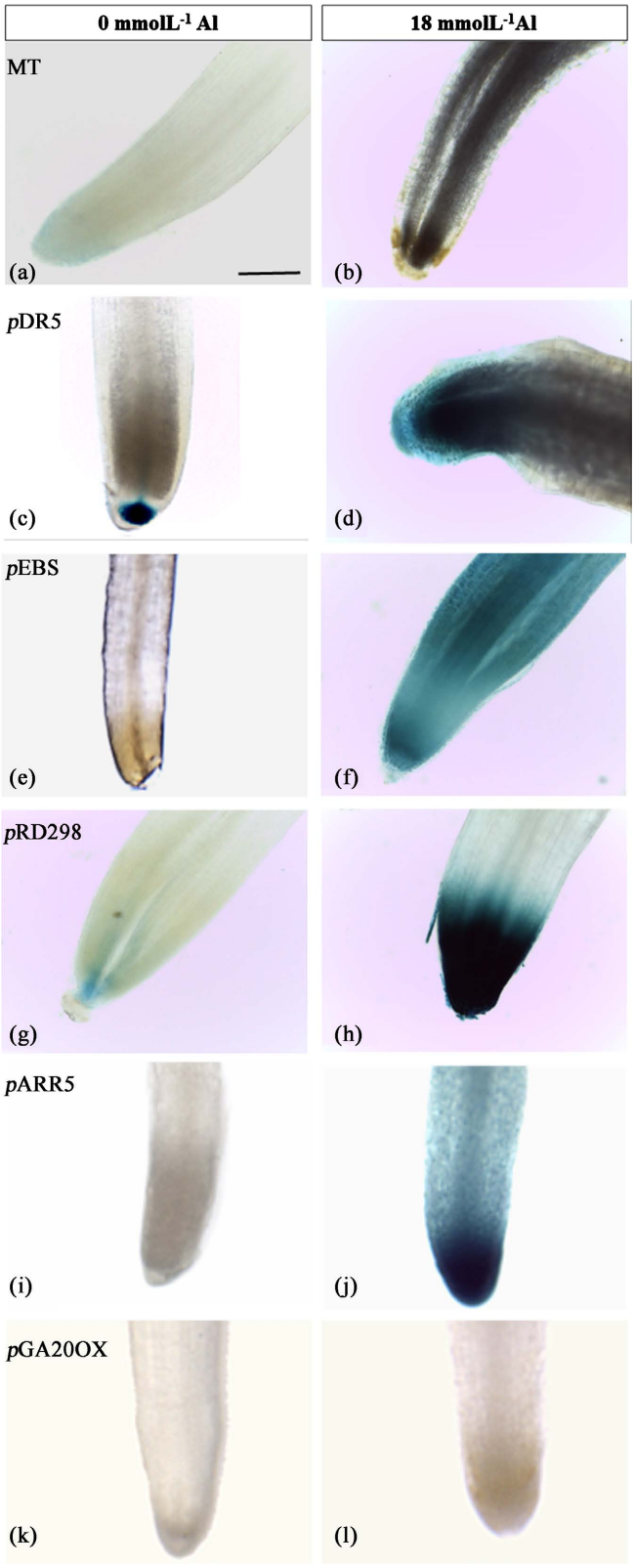

Figure 3. Effect of Al in activity of the GUS gene reporter in transgenic MT plants for different hormones, after 4 days of incubation with or without Al. (a)-(b) MT (Control); (c)-(d) DR5::GUS; (e)-(f) EBS::GUS; (g)-(h) RD29B::GUS; (i)-(j) ARR5::GUS and (k)-(l) GA2OX::GUS. Bars represent 250 μm.

show that, while AIA accumulates in the root apex, basipetal transport to the elongation zone is apparently inhibited. Therefore, Al exposure leads to an interference in normal AIA levels required for cell elongation specifically in the elongation zone. Al-treated transgenic plants expression of EBS::GUS, responsive to an ethylene pathway transcription factor (EIN3), was enhanced in the root tip and transition zone, albeit diffusely. Activity was also verified in the vascular tissue of the differentiation zone (**Figure 3(e)**, **Figure 3(f)**). In Al-treated plants, the ET response is generally seen as an upstream regulator of AIA synthesis in the root [11] [15]. Al-treated plants exhibit root elongation inhibition as well as an increase in ACC-oxidase activity [7], indicating that ET is directly involved in Al-induced root symptoms, including cell autophagy and apoptosis. Transgenic roots expressing RD29::GUS, responsive to ABA, demonstrated activity in the meristematic region in absence of Al (**Figure 3(g)**), but Al-treated roots showed diffuse expression in root tip cells, and to a lesser extent to the transition and elongation zones (**Figure 3(h)**). While ABA has generally been correlated with alleviation of Al toxicity, precise mechanisms by which this is achieved are not known [24]. However, a recent study correlates ABA with reactive oxygen species protection in Al-stressed plants [9]. Accordingly, ABA levels were highest in the root tip, which is most affected by Al toxicity, and would, then, have the highest levels of oxidative cell damage. Nonetheless, the protective mechanisms induced by ABA were not enough to prevent tissue necrosis and root growth inhibition (**Figure 1** and **Figure 2(a)**, **Figure 2(e)**). The expression of ARR5:GUS, which responds to CKs in the root tips, including both the meristem zone and transition zone, was highly induced under Al treatment (**Figure 3(i)**), even if less heterogeneously in the transition and differentiation zones than in the meristematic region (**Figure 3(j)**). In the transition zone, CKs seem to be the last step in root growth inhibition [25]. The authors show that CKs production is essential for diminishing root growth, since mutants deficient in CK biosynthesis exhibit less inhibition of root elongation in the presence of Al. In addition, GA accumulation was observed using plants expressing the GA2OX::GUS construct, which is responsive to GA2-oxidase activity. There was no GUS expression in either treatment, with (**Figure 3(k)**) and without Al (**Figure 3(l)**), after 4 days of Al exposure. GA2OX::GUS activity was also measured after 5 days of Al exposure, and the same negative results were obtained for both treatments without and with Al. There is relatively little information on the role of GAs in the Al response [24], however, it appears that GAs play a significant role in other stress responses, such as salt stress [26]. It is possible that a change in GA levels may only occur shortly after Al exposure, to elicit further responses from the root. In *Picea abies*, a GA peak in Al-stressed plants occurred after a 5 h treatment, but GA levels then decreased to sub-normal levels along nearly 9 months of treatment [27].

4. Conclusion

Altogether, the results emphasized the amplitude of hormone signaling changes

in the Al toxicity response of MT plants. Ethylene seems to be the primary mediator of root growth inhibition, inducing a signaling pathway which also directly involves AIA and ABA signaling, which, parallel to the ET pathway, may help alleviate metabolic stress. As seen with the results of GA levels, measuring hormone levels shortly after Al exposure is important to elucidate the precise roles and short-term effects of each hormone, and is the next step in unraveling the complex network of hormonal signaling involved in this stress response.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Wang, H.-H., Huang, J.-J. and Yu-Rong, B.-I. (2010) Nitrate Reductase-Dependent Nitric Oxide Production Is Involved in Aluminum Tolerance in Red Kidney Bean Roots. *Plant Science*, **179**, 281-288. https://doi.org/10.1016/j.plantsci.2010.05.014

[2] Rengel, Z. and Zhang, W.H. (2003) Role of Dynamics of Intracellular Calcium in Aluminum Toxicity Syndrome. *New Phytologist*, **159**, 295-314. https://doi.org/10.1046/j.1469-8137.2003.00821.x

[3] Arroyave, C., Tolrà, R., Thuy, T., Barceló, J. and Poschenrieder, C. (2013) Differential Aluminum Resistance in Brachiaria Species. *Environmental and Experimental Botany*, **89**, 11-18. https://doi.org/10.1016/j.envexpbot.2013.01.001

[4] Baluska, F., Mancuso, S., Volkmann, D. and Barlow, P.W. (2010) Root Apex Transition Zone: A Signalling-Response Nexus in the Root. *Trends in Plant Science*, **15**, 402-408. https://doi.org/10.1016/j.tplants.2010.04.007

[5] Silva, L.F.F., Lima, M.D.R., Lima, E.G.A., Castro, A.R.S., Barros Junior, U.O. and Lobato, A.K.S. (2017) Differential Behaviours in Two Species of Eucalyptus Exposed to Aluminium. *Indian Journal of Plant Physiology*, **22**, 107-113. https://doi.org/10.1007/s40502-017-0284-1

[6] Munde, N.A., Jadhao, K.R., Samal, K.C., Pradhan, S.K. and Rout, G.R. (2016) Allele Mining in Indica Rice (*Oryza sativa* L.) for ATP Binding Cassette (ABC) Transporter Gene Family for Aluminum Tolerance. *Indian Journal of Plant Physiology*, **21**, 161-170. https://doi.org/10.1007/s40502-016-0217-4

[7] Sun, P., Tian, Q.Y., Chen, J. and Zhan, W.H. (2010) Aluminium-Induced Inhibition of Root Elongation in Arabidopsis Is Mediated by Ethylene and Auxin. *Journal of Experimental Botany*, **61**, 347-356. https://doi.org/10.1093/jxb/erp306

[8] Yang, Z.-B., He, C., Ma, Y., Herde, M. and Ding, Z. (2017) Jasmonic Acid Enhances Al-Induced Root Growth Inhibition. *Plant Physiology*, **173**, 1420-1433. https://doi.org/10.1104/pp.16.01756

[9] Saha, I., Sarkar, B., Ghosh, A., Kumar, A. and Adak, M.K. (2019) Abscisic Acid Induced Cellular Responses of *Sub*1*A* QTL to Aluminium Toxicity in Rice (*Oryza sativa* L.). *Ecotoxicology and Environmental Safety*, **183**, Article ID: 109600. https://doi.org/10.1016/j.ecoenv.2019.109600

[10] Lima, J.E., Carvalho, F.R., Neto, A.T., Figueira, A. and Peres, L.E.P. (2004) Micro-MsK: A Tomato Genotype with Miniature Size, Short Life Cycle, and Improved *in Vitro* Shoot Regeneration. *Plant Science*, **167**, 753-757. https://doi.org/10.1016/j.plantsci.2004.05.023

[11] Ulmasov, T., Murfett, J., Hagen, G. and Guilfoyle, T.J. (1997) Aux/IAA Proteins Repress Expression of Reporter Genes Containing Natural and Highly Active Synthetic Auxin Response Elements. *Plant Cell*, **9**, 1963-1971. https://doi.org/10.1105/tpc.9.11.1963

[12] D'Agostino, I.B., Deruere, J. and Kieber, J.J. (2000) Characterization of the Response of the Arabidopsis Response Regulator Gene Family to Cytokinin. *Plant Physiology*, **124**, 1706-1717. https://doi.org/10.1104/pp.124.4.1706

[13] Dayan, J., Voronin, N., Gong, F., Sun, T.P., Hedden, P., Fromm, H. and Aloni, R. (2012) Leaf Induced Gibberellin Signaling Is Essential for Internode Elongation, Cambial Activity, and Fiber Differentiation in Tobacco Stems. *Plant Cell*, **24**, 66-79. https://doi.org/10.1105/tpc.111.093096

[14] Christmann, A., Hoffmann, T., Teplova, I., Grill, E. and Muller, A. (2005) Generation of Active Pools of Abscisic Acid Revealed by *in Vivo* Imaging of Water-Stressed *Arabidopsis*. *Plant Physiology*, **137**, 209-219. https://doi.org/10.1104/pp.104.053082

[15] Stepanova, A.N., Yun, J., Likhacheva, A.V. and Alonso, J.M. (2007) Multilevel Interactions between Ethylene and Auxin in Arabidopsis Roots. *Plant Cell*, **19**, 2169-2185. https://doi.org/10.1105/tpc.107.052068

[16] Watanabe, T. and Osaki, M. (2001) Influence of Aluminum and Phosphorus on Growth and Xylem Sap Composition in *Melastoma malabathricum* L. *Plant and Soil*, **237**, 63-70. https://doi.org/10.1023/A:1013395814958

[17] Wang, Y., Li, R., Li, D., Jia, X., Zhou, D., Li, J., Lyi, S.M., Hou, S., Huang, Y., Kochian, L.V. and Liu, J. (2017) NIP1;2 Is a Plasma Membrane-Localized Transporter Mediating Aluminum Uptake, Translocation, and Tolerance in Arabidopsis. *Proceedings of National Academy of Sciences*, **114**, 5047-5052. https://doi.org/10.1073/pnas.1618557114

[18] Schneider, C.A., Rasband, W.S. and Eliceiri, K.W. (2012) NIH Image to ImageJ: 25 Years of Image Analysis. *Nature Methods*, **9**, 671-675. https://doi.org/10.1038/nmeth.2089

[19] Yamamoto, Y., Kobayashi, Y. and Matsumoto, H. (2001) Lipid Peroxidation Is an Early Symptom Triggered by Aluminum, but Not the Primary Cause of Elongation Inhibition in Pea Roots. *Plant Physiology*, **125**, 199-208. https://doi.org/10.1104/pp.125.1.199

[20] Motoda, H., Kano, Y., Hiragami, F., Kawamura, K. and Matsumoto, H. (2011) Changes in Rupture Formation and Zonary Region Stained with Evans' Blue during the Recovery Process from Aluminum Toxicity in the Pea Root Apex. *Plant Signaling & Behavior*, **6**, 98-100. https://doi.org/10.4161/psb.6.1.14148

[21] He, H., Li, Y. and He, L.F. (2019) Aluminum Toxicity and Tolerance in Solanaceae Plants. *South African Journal of Botany*, **123**, 23-29. https://doi.org/10.1016/j.sajb.2019.02.008

[22] Chen, Y., Yordanov, Y.S., Ma, C., Strauss, S. and Busov, V.B. (2013) DR5 as a Re-

porter System to Study Auxin Response in Populus. *Plant Cell Reports*, **32**, 453-463. https://doi.org/10.1007/s00299-012-1378-x

[23] Li, C., Liu, G., Geng, X., He, C., Quan, T., Hayashi, K.-I., Smet, I.D., Helene, S.R., Ding, Z. and Yang, Z.-B. (2021) Local Regulation of Auxin Transport in Root-Apex Transition Zone Mediates Aluminium-Induced Arabidopsis Root-Growth Inhibition. *Plant Journal*, **108**, 55-66. https://doi.org/10.1111/tpj.15424

[24] Kopittke, P.M. (2016) Role of Phytohormones in Aluminum Rhizotoxicity. *Plant, Cell & Environment*, **39**, 2319-2328. https://doi.org/10.1111/pce.12786

[25] Yang, Z., Liu, G., Liu, J., Zhang, B., Meng, W., Muller, B., Hayashi, K.I., Zhang, X., Zhao, Z., Smet, I. and Ding, Z. (2017) Synergistic Action of Auxin and Cytokinin Mediates Aluminum-Induced Root Growth Inhibition in Arabidopsis. *EMBO Reports*, **18**, 1213-1230. https://doi.org/10.15252/embr.201643806

[26] Lv, S.F., Yu, D.Y., Sun, Q.Q., *et al.* (2018) Activation of Gibberellin 20-Oxidase 2 Undermines Auxindependent Root and Root Hair Growth in NaCl-Stressed *Arabidopsis* Seedlings. *Plant Growth Regulation*, **84**, 225-236. https://doi.org/10.1007/s10725-017-0333-9

[27] Cizkova, R. (1995) Phytohormonal Levels in Spruce Roots under Aluminium Stress. In: Baluska, F., Ciamporova, M., Gasparikova, O. and Barlow, P.W., Ed., *Structure and Function of Roots*: *Developments in Plant and Soil Sciences*, Springer, Berlin, 335-339. https://doi.org/10.1007/978-94-017-3101-0_44