Effect of Aluminum Stress on Onion (Allium cepa L.)

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ABSTRACT

This study analyzed the effect of Aluminium (Al) on onion (Allium cepa) root growth and its defense response. The experiment was carried out by exposing onion to 10, 100, 500 and 1000 µM AlCl\(_3\) for 24, 48 and 72 hours. Parameters observed were root length and diameter, root tip mitotic index, cortical parenchyma cell length and cortical cell layer thickness. Onion defense response observed by measuring malic, citric and oxalic acid quantitatively, and the accumulation of Al and callose in root qualitatively. Results showed that an increase in the concentration of Al caused a decrease in root length. At concentrations up to 100 µM, Al increased root diameter and cell length whilst at higher concentrations, Al decreased both parameters. Compared to control, all Al-treated root had lower index mitosis with 10 µM Al-treated root displayed highest IM. Since root treated with Al higher than 10 µM showed none or very low mitotic activity, chromosome aberration can only be observed in 10 µM Al-treated roots. Root defense response via Al and callose accumulation was increased with the increasing of Al concentrations. Organic acids were present in higher amounts in root than in medium with a ratio of 5000:1. Oxalic acid appeared to be the major organic acid that played a role in defense mechanisms against Al stress. Based on the results, it can be concluded that (1) Al\(^{3+}\) were toxic to Allium cepa because it caused root growth inhibition and chromosomal aberrations; (2) defense response for Al\(^{3+}\) stress were via the increase of callose as well as Al accumulation and organic acids content in root cells.

Keywords: Allium cepa, aluminum, defense response

INTRODUCTION

Aluminum (Al) is an element commonly found in earth’s surface. In neutral condition (pH 7), it is stable, insoluble and non reactive [3]. In acidic condition, it is in the form of Al\(^{3+}\) ions and soluble in water. It also can be reactive and toxic [19]. It can cause interference to the cell wall, plasma membrane and a variety of physiological processes in the cell. The toxicity initially occurs at plant root tip [11] and generally, the roots will experience growth inhibition due to inhibition of cell elongation [22] and/or cell division indicated by the decrease in mitotic index [8].

Plants have two types of defense mechanism for Al stress, that are organic acid exudation or accumulation of Al which has been detoxified by organic acids [10]. Organic acids are exuded by roots to bind Al ions in rhizosphere so that they cannot enter the roots. However, when Al ions are able to enter the root, they will then be neutralized by organic acids to form Al-organic acid complex. These complexes are accumulated in leaf vacuoles so they are no longer be toxic to plant [16]. Usually this kind of defense response is found in plants with high degree of tolerance towards Al.

EXPERIMENTAL

This study aims are to analyze the effect of Al on onion (Allium cepa) root growth and its defense response to overcome Al stress.

Aluminium stress treatment

Al stress was given by placing rooted onions in containers containing different concentrations of AlCl\(_3\) (10, 100, 500 and 1000 µM) with pH 4.5. Distilled water with neutral pH was used as a control. The experiments were performed in dark condition at room temperature, and the medium was changed every day. Effect of AlCl\(_3\) on root growth was observed after 24, 48, and 72 hours by measuring root length and diameter, cortical parenchyma cell length and cortical cell layer thickness and root tip mitotic index.

Onion Root Tip Mitotic Index

Root tip mitotic index is counted after making squash slides with Carnoy fixative and acetocarmine dye [2]. Around 2 mm from the root tip are cut, stained and squashed in preparation slide. Mitotic index is a number of cell in mitotic stage per total cell observed (1000 cells).
Observation of Defence Response

Al accumulation analysis performed on fresh root samples stained with hematoxylin [1], then observed under a light microscope. Accumulated callose analyzed with aniline blue staining and observed under a fluorescence microscope. Analysis of organic acids from both onion root and medium performed after 24- and 72-hours treatment using Tian et al. method [23]. Samples were analyzed using HPLC with KH2PO4 0.01 mL/L (pH 2.6) as eluent buffer with flow rate of 1 mL/min. The absorbance of organic acids detected at wavelength of 215 nm.

RESULTS AND DISCUSSION

Effect of Aluminium on Root Growth

Parameters observed to determine the effect of Al on root growth were root length and diameter, root tip mitotic index, cortical parenchyma cell length and cortical cell layer thickness.

Effect of Al on root length, root diameter, root tip mitotic index and the length of cortical parenchyma cells can be seen in Figure 1-3. Figure 1 showed that an increase in Al concentration caused a decrease in root length from 14% to 23% decrease. However, the Al effect on root diameter was rather different (Figure 2A). When compared with the effect on root diameter and cortex layer thickness, it appears that Al have different effects on root length and root diameter. At concentrations less than or equal to 100 µM, Al causes an increase on root diameter (11% for 10 µM and 4 % for 100 µM) and cortex layer thickness (9% for 10 µM and 6% for 100 µM), but concentration greater than 100 µM will decrease both (Figure 2A and B). The root diameter decreased to 5% and 20% from 500 µM and 1000 µM treatment respectively and cortex layer thickness decreased 3% and 15% from 500 µM and 1000 µM treatment. The influence of Al on root length follows a regular pattern in which an increase in the concentration of Al causes a decrease in root length (Figure 1). Thus, root-treated Al up to 100 µM will be shorter and stubbier than the control, while higher Al concentration causes the roots to be short and ‘thin’.

The effects of Al on root tip mitotic index are displayed in Figure 3A. It can be seen that all Al-treated roots had lower MI compared to control, with the lowest MI (0 – 0.5%) shown by all roots treated with concentration greater than 10 µM. In those roots, the cells either had no cell division activity or if there was an activity, the frequency was very low.

Al effects on the cortical parenchyma cells length showed two different patterns (Figure 3B). At high concentrations of 500 and 1000 µM, Al caused a decrease in cell length at 22% and 25 % respectively at the 72 hours treatment and the decrease in line with the treatment duration. At lower concentrations, that were 10 and 100 µM, the cell length increased so that eventually they had approximately similar length as control cells.

Figure 1. Effect of aluminium with different concentrations (10, 100, 500 and 1000 µM) on root length after 0, 24, 48 and 72 hours of treatment.

Al detrimental impact on the genetic material and/or structure that is associated with genetic material such as spindle thread can also be observed in this study. However, the impacts can only observed in cells of 10 µM Al-treated roots in the form of sticky chromosome aberration (Figure 4A and 4B) and aberration caused by spindle disruption (Figure 4C). The presence of chromosomal aberrations which only found in 10 µM Al-treated roots can due to very low (0-0.5%) mitotic index in

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higher Al concentration-treated roots. Only the cell with mitotic activity will have the possibility to undergo the chromosome aberration.

Figure 2. Effect of aluminum with different concentrations (10, 100, 500 and 1000 µM) on root diameter (A), cortex layer thickness (B) after 0, 24, 48 and 72 hours of treatment.

Another detrimental impact of Al on genetic material observed in this study is cells with binuclei. The effect of Al to the number of root cells with binuclei can be seen in Figure 5. It appears that at concentrations below 1000 µM, Al effect was not significant, but as treatment continues, an increase in the number of binuclei observed.

Figure 5. Effect of aluminum with different concentrations (10, 100, 500 and 1000 µM) to the number binuclei formed in onion roots treated for 24, 48 and 72 hours.

We also found necrotic cells on onion roots treated with Al (Figure 6). Effect of Al on the number of necrotic cells showed an increase along with the increasing concentration of Al given but significant increase occurred at treatment group with Al concentration above 10 µM (Figure 7). Necrotic cell observed after 72 hours treatment increased 8.22, 7.67 and 9.56 times for 100 µM, 500 µM and 1000 µM treatment respectively. Figure 7 also shows that the effect of Al on necrotic cells is dose-dependent and not time-dependent.

Figure 6. Necrotic cell found in onion root (arrow)
Figure 7. Effect of aluminum with different concentrations (10, 100, 500 and 1000 µM) on onion root necrotic cells treated for 24, 48 and 72 hours.

Aluminium and Callose Accumulation in Onion roots

Figure 8 displayed the effect of Al on Al accumulation in onion root. It can be seen that higher Al and longer treatment caused the root colour became more intense. The increase of colour indicated the amount of AIPO₄ that stained with haematoxylin and implied the amount of Al in the root [21]. Callose accumulation in all Al-treated onion roots shown by luminescence on the roots when observed under fluorescence microscope. It appears that increasing concentration of Al increasing root fluorescence (Figure 9), thus, the higher Al given, the higher callose deposited.

Organic Acid Concentration in Root and Growing Medium

Organic acids analyzed in this study were citric, malic and oxalic acid. The concentration of these organic acids in the onion root and growing medium can be seen in Figure 10. It appeared that all organic acids found in roots were higher than in medium with ratio of 5000 : 1 (root : medium). The concentration of oxalate and citrate in the medium and roots showed a time-dependent pattern. Although, the time-dependent pattern was contradictory. Over time, those two organic acids appeared to increased in root, but decline in the medium. In contrast to those organic acids, malic acid that detected in root and medium exhibited huge variation pattern. Malic acid levels were not either dose-dependent or time-dependent.

Figure 8. Effect of aluminum with different concentrations (10, 100, 500 and 1000 µM) for 24 and 72 hours on aluminum accumulation in onion root tip.

Figure 9. Effect of various concentrations of aluminum on callose deposit on onion root (A) control, (B) 10, (C) 100 (D) 500 and (E) 1000 µM.

Figure 10. Effect of aluminum with different concentrations on organic acids concentration in root treated for 24 and 72 hours.
that organizes chromosomes during cell division. Al ability to inhibit the formation and also decomposition of spindle thread lead to disruption of spindle thread function. The disruption caused chromosome disorganization when mitosis progressed. At low concentrations (18 to 55 µM) and in long exposure (48 hours), Al can enter the cell and bind to cell nucleus [24]. This event leads to the stabilization of the chromatin condensation and inhibition of cell division activity [17]. The effect of Al on cell length is possibly because Al can affect factors that directly or indirectly related to the process of cell elongation, i.e. by affecting the organization of the cytoskeleton, the levels of Ca$^{2+}$ in the cell and the composition of the plasma membrane and cell wall composition. Cytoskeleton has a role in determining the direction of cell elongation [25] and is required for cell wall formation [6]. Al can also replace Ca$^{2+}$ on pectin so that the matrix cannot be stretched and so that the cell walls become rigid and cannot elongate [5].

Al effects on cortex layer thickness showing the same pattern with root diameter. Increase in root diameter could be caused by increase in cortex layer thickness. According to Ciamporova [9], increase in root diameter treated with Al caused by cortex cell uneven radial growth. Al could disrupt cytoskeleton organization and caused disorder in cell expansion orientation that leads to uneven cortex cell uneven radial growth. It could be concluded that the change in the diameter of the root treated with Al caused by changes in the cortex layer thickness.

Aside from Al effect on MI, Al also caused chromosome aberration such as sticky chromosome and aberration due to spindle disruption. Sticky chromosomes indicate a direct interference in the DNA that causes the chromosomes attached to each other. When Al attached to the histone protein or phosphate group, it caused the DNA condensed [18]. The aberration due to by spindle disruption showed that Al affects microtubules, the proteins that form the structure of the spindle thread.

In addition to chromosomal aberrations, we also found binuclei cells. In these cells, cytokinesis does not occur after mitosis so that the two nucleus remains in the same cell. Frantzios et al. [12] reported that cytokinesis inhibition can be caused by a disturbance in the process of phragmoplast formation, namely structure compiled by microtubules [20] and serves as the framework in the formation of new cell wall.

Effect of Al on onion root tip cells also indicated by the presence of cells that undergoing necrosis or necrotic cells. According Lockshin and Zakeri [14]. These cells undergo unprogrammed/uncontrolled death process due to extreme stress by external factors. Necrotic cell characterized by loss of plasma membrane integrity resulting in cell swelling and release of cell contents [7]. Loss of plasma membrane integrity caused by the attachment of Al on the plasma membrane which caused the plasma membrane becomes rigid. Rigid plasma membrane will lose its flexibility thus more easily damaged. The binding of Al to the plasma membrane can also

CONCLUSION

Al toxicity on onion root growth were observed through growth parameters such as root length and diameter, root tip mitotic index, cortical parenchyma cell length and cortical cell layer thickness. The elongation of root can be caused by any process of cell division or cell elongation alone or cell division that accompanied by cell elongation [4]. In this experiment, it seems that the inhibition of root elongation treated with Al occurred because of cell division and cell elongation inhibition. The inhibition of root length in this experiment which was due to a reduction in the mitotic index, was also observed by Campos and Viccini [8].

Al in cells can bind to DNA and interrupt the process of mitosis and cells remain in interphase stage [18]. Inhibition effect of Al on root tip cell division activity can also be due to disruption of microtubule function [12]. It is known that microtubules play a role in the formation of the spindle thread

Figure 11. Effect of aluminum with different concentrations on organic acids concentration in growth medium treated for 24 and 72 hours

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<th>Oxalate</th>
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<td>Concentration (ppm)</td>
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reduce membrane permeability to water. It has been reported that Al can cause oxidative stress that causes damage to the various components of the cell, including the plasma membrane [27]. Research conducted by Campos and Viccini [8] on onion (Allium cepa) showed an increased cell death when treated with Al solution higher than 100 μM. The difference may due to (1) differences in Al used (Al₂SO₄ vs AlCl₃) and (2) different source of onions used.

Most of soil Al can be found in aluminosilicate form which is insoluble and non-reactive. Only few soluble Al ions are toxic. The most toxic Al in ion form is Al³⁺ which can be found plenty in acidic condition [19]. When plants were exposed to soluble Al ion, Al was able to enter the root cells via calcium channels or nonspecific cation channels. It will then react with an inorganic phosphor ion to form AlPO₄. These ions are released by cells when plants experience stress. The AlPO₄ precipitation and deposition in outermost cells of the root cortex will be stained by hematoxylin [21]. Increasingly dense colour indicates the increase of AlPO₄ which stained by hematoxylin and implies the amount of Al in root.

Induction of callose (-1,3-glucan) formation is a sensitive marker for genotypic Al toxicity (Horst et al. 1997). Callose is accumulated in the cell wall around plasmodesmata in response to the damage caused by Al in the roots of various plants. Larsen et al. [13] observed increasing callose deposition in wild-type Arabidopsis seedling roots with increasing Al concentrations over the range of 0 to 100 μM AlCl₃. Callose may cause the blockage of cell–to-cell transport by blocking plasmodesmata [22] and the growth of onion root in this experiment will was also hampered.

Among the three organic acid measured, the concentration of oxalic acid was the highest (5000-25000 ppm) or 10 times compared to the other, whilst malic acid was the lowest. There is a possibility of oxalic acid is the main organic compound produced by onion as defence response against Al stress [15].

During experiment, malic acid concentration showed enormous variation and irregular pattern. This may due to the defence response via malic acid was more than 72 hours or the onion did not use malic acid as a defence response to the stress of Al so there is no correlation between malic acids level with Al treatment [26]. Organic acid concentrations found in roots are higher than in medium with a ratio of 5000:1. It may show that onion roots prefer to detoxify Al inside the roots with organic acid as defence response.

From the results obtained, it can be concluded that Al inhibited root growth through cell division and elongation. Al also caused chromosomal aberrations and cell necrosis. The higher concentration and the longer exposure to Al, Al that enter and accumulate in the roots also increased. As a defence reaction, onion produced callose and organic acids, especially oxalic acid.

ACKNOWLEDGEMENT

REFERENCES


