Chapter 4
Aluminum Uptake, Callose Accumulation, and Invertase Activity in Lowland and Upland Rice Genotypes in Relation to Aluminum Stress Tolerance

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Abstract Aluminum is among the prominent restricting factor accountable for the growth of plants in acidic soils pertaining to its solubility. The absorption of aluminum (Al^{3+}) by rice roots was examined in this work using seedlings from two cultivars (tolerant and sensitive) treated with $AlCl_3$ (1 mM) in fresh sand culture. The progression of Al through the roots was seen using hematoxylin and Eriochrome cyanine R staining. When compared to the sensitive cultivar, the tolerant cultivar has a reduced Al absorption rate. It shows the presence of some extrinsic mechanisms that control the tolerant rice cultivar's behavior. The increase in Evans blue absorption after Al^{3+} treatment indicated root cell injury, with more apparent uptake in sensitive cultivars than tolerant cultivars. Aluminum fluorescence intensity increased with Morin staining in sensitive than tolerant cultivars, according to confocal microscopy. The results presented here confirmed that the increased accumulation of Al^{3+} leads to reduced rice seedlings growth and increased invertase activity, callose accumulation, and cell death. It also indicates that metabolic procedures and the transduction of the signals contributing to increased invertase movement help the tolerant variety maintain higher root development in harmful Al^{3+} concentrations.

Keywords Aluminum toxicity · Al localization · Confocal · Rice (*Oryza sativa* L.)

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A. Kumar et al. (eds.), *Agriculture, Livestock Production and Aquaculture*,
https://doi.org/10.1007/978-3-030-93258-9_4

1 Introduction

One of the foremost contributors and constraints contributing to crop growth in acidic soils is aluminum (Al) phytotoxicity that accounts for 40 % of arable land (Sade et al. 2016). This phytotoxicity is found to be released in big quantities at low pH (pH 5.0 or less). This led to damage to overall root structure, growth, and thus yield. Al has a complex chemical structure as well as biological activities (Kochian et al. 2005). The pH scale of the soil determines the Al that is available in the soil. The structure is octahedral hexahydrate, Al $(H_2O)_6^{3+}$, at low pH (about 4.3), and is sometimes abbreviated as Al^3. The problem of Al^{3+} toxicity is multifarious through soil acidification caused due to inconsistent usage of ammonia as well as fertilizers constituting amides. Nitrogen fixation in legumes, an imbalance between nitrogen and sulfur (Guo et al. 2010), and absorption of excess cations and anions also contribute to this acidification. Al is not just phytotoxic but inhibits plant growth too. Besides, it also harms human health because it is passed down the food chain from animals to people (Jaishankar et al. 2014). The key indication of toxicity caused by Al is suppression in the growth of the roots that usually happens as soon as 1 or 2 h after Al exposure (Horst et al. 2010). This root growth suppression results in a thin, thickened, diminished, and uneven root system (Ciamporova 2002). The variants that are Al sensitive tend to gather additional Al within the root tissue than Al tolerant. For example, Al-sensitive cultivars like maize, rice, wheat, and soybean displayed more Al contents in their root tips than the resistant version (Du et al. 2010; Kang et al. 2011; Matonyei et al. 2014; Garcia-Oliveira et al. 2016).

The progression and build-up of the Al in the root tissues could be measured using a variety of methods. The employment of Al-specific dyes is one of them. This build-up is required to be investigated for which staining techniques are often quick and easy. One of the most common methods is the comparison of the length and weight of the roots of the plants affected with or without Al. Nonetheless, this method is not ideal for the large population as there are significant variations in root length, and this process takes time also. Because of these drawbacks, a quick method, *staining with hematoxylin*, was discovered that utilizes the coloring formation when the Al-induced root with the stain (Polle et al. 1978). A few researchers have used this method to screen Al resistance phenotypes in various crop species, such as maize (Lidon et al. 2007), pea (Singh et al. 2009), and chickpea (Singh and Chaturvedi 2007). Another method, *staining with Eriochrome cyanine R*, enables the visualization of the Al across the root. In this, the roots damaged because of Al were deeply stained (pink) despite non-damaged roots remaining unstained (white). This method is suitable for assessing large numbers of plants at the same time. *Evans blue staining* is considered as an index of damages caused by plasma membranes and programmed cell death in plants (Yamamoto et al. 2001). Minute concentrations of Al could be identified using fluorophores like Morin using fluorescence microscopy (Eticha and Horst 2005). The detection limit has been identified as 2×10^{-9} M. (Lian et al. 2003).

Along with the inhibition of Al-induced growth of the root, the induction of callose formation was observed in several plant species as a physiological pointer to the Al sensitivity (Jones et al. 2006). Different stressors are identified as the triggering agent for callose synthesis whose in-depth methodology is unknown but compromises the plasma membrane's integrity and function (Chen and Kim 2009). In biotic and abiotic stress conditions, an increase in cytosolic calcium (Ca^{2+}) activity synthesizes the β-D-glucan in plants (Bhuja et al. 2004). Al also causes a transient upsurge in cytosolic Ca^{2+}, according to Bhalerao and Prabhu (2013).

In abundance, metals such as Cd^{2+}, Ni^{2+}, Al^{3+}, and As^{3+} induce toxicity in growing plants and also influence the activity behavior of various carbohydrate metabolism-related enzymes (Verma and Dubey 2001; Mishra and Dubey 2013; Jha and Dubey 2004; Mishra and Dubey 2008; Abbas et al. 2018). The main photosynthetic end products are starch and sugar, and their levels inside plants give important plant production information. Under diverse stress conditions, starch and sugar storage, translocation, and metabolism are all impacted. The metabolism of sucrose is critical for energy production and plant growth under stressful conditions (Harada et al. 2005). Invertases are found in enzymes that regulate the sucrose content in plants in acidic, alkaline, and neutral forms (Tauzin and Giardina 2014). Sucrose-aided root cell division, elongation, and expansion. As a result, Al^{3+}-induced changes in invertase activity are probably part of a cell-wall-based toxicity resistance mechanism to aluminum. The most Al-resistant cereal crop is rice which uses several methods to achieve high Al resistance. Little is known on the mechanism of increase in Al tolerance in rice (Famoso et al. 2010). It promoted us to use rice as a model plant to examine the mechanism of Al tolerance.

To explore this phenomenon in detail, two rice cultivars have been used, namely, Malviya-36 as the Al-sensitive one and Vandana as the Al-tolerant one. It has been observed that distinct Al^{3+} concentration in roots generally leads to varying tolerance levels, and thus, invertase activity and callose accumulation have been examined as disclosed by Morin stain.

The selection of appropriate cultivars is essential for the study of tolerance mechanisms. For this, the sensitivity and tolerance mechanism of both cultivars was confirmed using *Eriochrome cyanine R staining*. Al uptake in roots is seen by *hematoxylin staining* in different cultivars to separate the degree of participation for exploring the tolerance mechanism at the external as well as internal levels.

2 Materials and Methods

2.1 Plant Material and Treatment Levels

Malviya-36 and Vandana are the two variants of Indica rice (*Oryza sativa* L.) cvs. seeds that are used for conducting the experimental work. After a 24-hour inhibition in water, seeds are wet with 0.5 mM $CaCl_2$ solution and kept at 28±1°C with 80 %

relative humidity in the BOD incubator cum humidity (York Scientific Industries, New Delhi, India) for 5 days. Seedlings subsequently were elevated in purified quartz sea sand, and Yoshida nutrient solution is used for the next 10 days for saturation (Yoshida et al. 1976). Further, the uprooted seedlings were treated with $AlCl_3$ (1mM) for the next 2 days in the presence of fresh and sand cultures.

2.2 The Impact of Morin Staining in the Localization of Al in Roots

Morin staining as defined by Tice et al. (1992) has been utilized in the current work. The roots were initially washed for 10 min in the presence of a buffer, NH_4OAc having a pH of 5.5, followed by staining by 100 μM for 1 hour in the same buffer. Till the elution of the stains, washing will be continued. The lateral segments of the roots were cut and then observed through a microscope coupled with fluorescence at an excitation and an emission wavelength of 420 and 510 nm, respectively, until it fluoresced green.

2.3 Hematoxylin Staining in the Detection of the Accumulated Al in Root Tips

Hematoxylin staining (Ownby 1993) has been utilized for visualizing the deposition of the Al in the roots of sensitive as well as tolerant variants of the cultivars. The roots were initially washed for 15 min followed by staining at room temperature. Staining is done using a solution of 0.2% hematoxylin (w/v) and 0.02% KIO_3 (w/v). Further, distilled water is used for washing it for 15 min, and then photographs were taken. A spectrophotometer (Bausch and Lomb, Spectronic 20, USA) was utilized for quantifying the optical density of the stains at 490 nm on 15 root tips of 5 mm each after 1ml of 1 M HCL treatment for 1 hour.

2.4 Determination of Loss of Plasma Membrane Integrity

The loss of integrity of the plasma membranes has been determined using the modified Evans blue staining method as defined by Schützendübel et al. (2001). The staining of the seedlings was conducted for half an hour with a 0.25% (w/v) aqueous solution. The washed cross-sectional root tips with distilled water were utilized for viewing through the light microscope. To determined Evans Blue Uptake, stained cross-sections roots of 10 mm length were homogenized in 1 ml of 1% (w/v) aqueous sodium dodecyl sulfate (SDS) followed by centrifugation at

13,500 × g for 10 min at room temperature. Measure the optical density of the supernatant spectrophotometrically at 600 nm.

2.5 Eriochrome Cyanine R Staining

To confirm the sensitivity and tolerance of selected rice cultivars toward Al toxicity, Eriochrome cyanine R staining was used (Aniol 1995). Seedlings were removed and placed in distilled water for 30 min after 48 h of Al treatment. One-tenth of an aqueous solution of Eriochrome cyanine R was used to stain the roots for 10 min. The surplus dye was detached by several washes with distilled water, and then photographs were taken with a high-resolution camera.

2.6 Histochemical Detection of Callose in Root Tissues

The Al^{3+}-induced callose accumulation detection has been conducted using aniline blue staining in the current work. The roots are secured for 1 h using 10% ethanol (FAA) and formaldehyde and 5 % glacial acetic acid. The top 1 cm root tip was cut and cleaned using de-ionized water and stained for 10 min (Kauss 1992). One-tenth of water-soluble aniline blue is applied to 50 mM glycine-NaOH buffer with a pH of 9.5 and then explored using a fluorescence microscope.

2.7 Measurement of Callose Content

The calculation of callose content has been done according to Bhuja et al. (2004). A total of 25 root segments were taken and washed in ethanol for 30 min followed by homogenization in 1M NaOH. The samples were incubated at 80 °C for 20 min and further centrifuged at 12,000 × g for 5 min. The resultant was further treated to the following combination.

- 50 µl of supernatant along with 100 µl of 0.1% aniline blue
- 50 µl of 1M HCl
- 150 µl of glycine-NaOH buffer pH 9.5

Afterward, incubation for 20 min at 50 °Cand 30 min at room temperature fluorescence signal recorded using excitation at 380 nm (filter 530/20) and detection of fluorescence at 485 nm (filter 485/20).

2.8 Analysis of Invertase Activity

A total of six seedlings of each Al^{3+} exposed cultivar is incubated for 3 h to detect invertase activity. These seedlings were treated to a neutral reaction medium buffer at room temperature; after which, photographs were captured using Olympus light microscope. The buffers (Doehlert and Felker 1987; Zrenner et al. 1995) consisted of the following solutions:

- 0.38 mM sodium phosphate with pH of 7.5
- 0.024% tetrazolium blue
- 0.014% phenazine metosulfate
- 30 U of glucose oxidase
- 30 mM of sucrose
- For control, incubation medium without sucrose and Glucose oxidase

2.9 Statistical Analysis

The experimentations were repeated thrice, and the data was used as the mean of the standard deviation of the obtained replicates. Analysis of variance (ANOVA) is used for obtaining the mean difference of the control and treatment groups. The significance of this difference is represented as * for $p \leq 0.05$ and ** for $p \leq 0.01$. Further, Tukey's multiple range test has been used.

3 Results

3.1 The Impact of Morin Staining in the Localization of Al in Roots

Morin (3,5,7,2′,4′-pentahydroxyflavone) is an Al-specific fluorescent dye that constitutes a fluorescent complex when combined with Al. The greater the Al accumulation, the brighter the fluorescent light. An intense green Morin fluorescence was seen in the root section, whereas there was almost little fluorescence in control (–Al) plants (Fig. 4.1). It was observed that low fluorescence was found in roots stained with Morin that was unexposed to Al and whatever fluorescence was seen was confined to the epidermal surface. When compared to sensitive roots, tolerant cultivar root cross-sections revealed significantly less Morin staining.

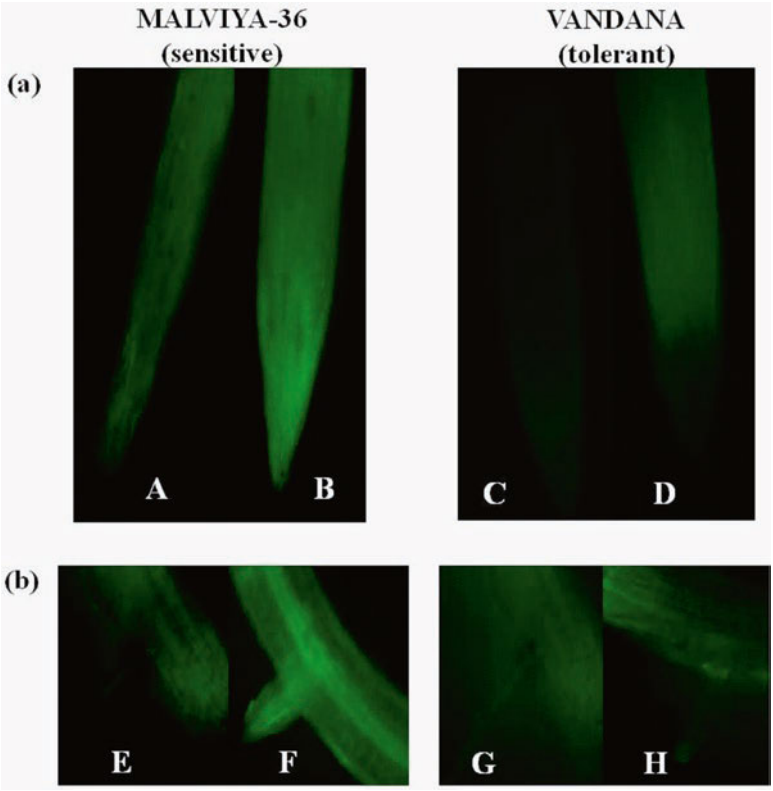

Fig. 4.1 (**a**) Fluorescence detection of Al in rice root tips after Morin (100 μM) staining in roots of Malviya-36 and Vandana seedlings. The Seedlings were grown for 10 days and then uprooted followed by treatment either in Yoshida nutrient solution (control, without Al^{3+}, denoted as *A, C*) or in nutrient solutions containing 1mM Al^{3+} (*B*, *D*) for 48 h. **(b)** The lateral roots of the seedlings obtained from matured segments of the primary roots

3.2 Determination of Aluminum Uptake

A hematoxylin stain was utilized to investigate the uptake of Al in the seedlings' roots. When the roots of cvs. Malviya-36 and Vandana seedlings are stained with the Al-specific dye hematoxylin, it is detected that the roots of Al-treated seedlings of Malviya-36 take up a greater amount of stain than tolerant cv. Vandana (Fig. 4.2). Though there was no reaction with Al^{3+}, the hematoxylin stain was absorbed by control roots, and thus dull redness is visible. Spectrophotometric analyses revealed that dye uptake was increased by four- and twofold in roots of 1mM Al^{3+}-treated seedlings in cv. Malviya-36 and cv. Vandana, respectively, as compared to controls.

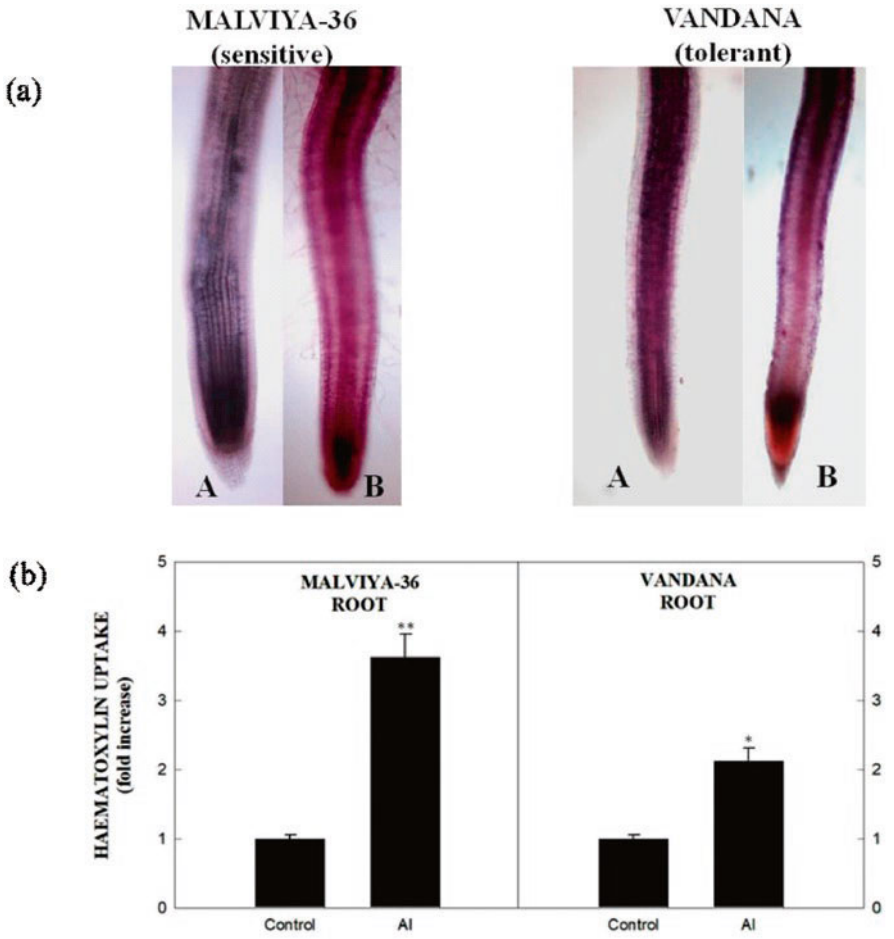

Fig. 4.2 **(a)** Microscopic view of hematoxylin-stained roots of Malviya-36 and Vandana seedlings. The seedlings were grown in sand cultures for 10 days followed by its treatment in either Yoshida nutrient solution (control, without Al^{3+}, denoted as *A*) or in nutrient solutions containing 1mM Al^{3+} (*B*) for 48 h. **(b)** The exposure of hematoxylin staining resulted in the accumulation of Al in root tips (5 mm). The resultant value after three independent values is mean ± SD (standard deviation depicted by bars). As per Turkey's test, * and ** depict the differences as per controls at $p \leq 0.05$ and $p \leq 0.01$, respectively

3.3 The Damage in a Membrane Formed Due to Al in Root Tips

Evans blue has been utilized as a marker for cell death as well as for identifying the loss in the integrity of the plasma membrane. An increase of Evans blue absorption was detected in comparison to controls for Al-treated rice seedlings. Evans blue

stain uptake increased significantly ($p \leq 0.01$) in the roots of both rice cultivars as the concentration of Al^{3+} treatment increased, with notably higher uptake in the Al sensitive cultivar than the tolerant cultivar (Fig. 4.3). Even in controls, the stain taken by roots of cv. Malviya-36 seedlings were higher than cv. Vandana. The findings make a strong point regarding the increased permeability of the plasma membranes after treating them with Al.

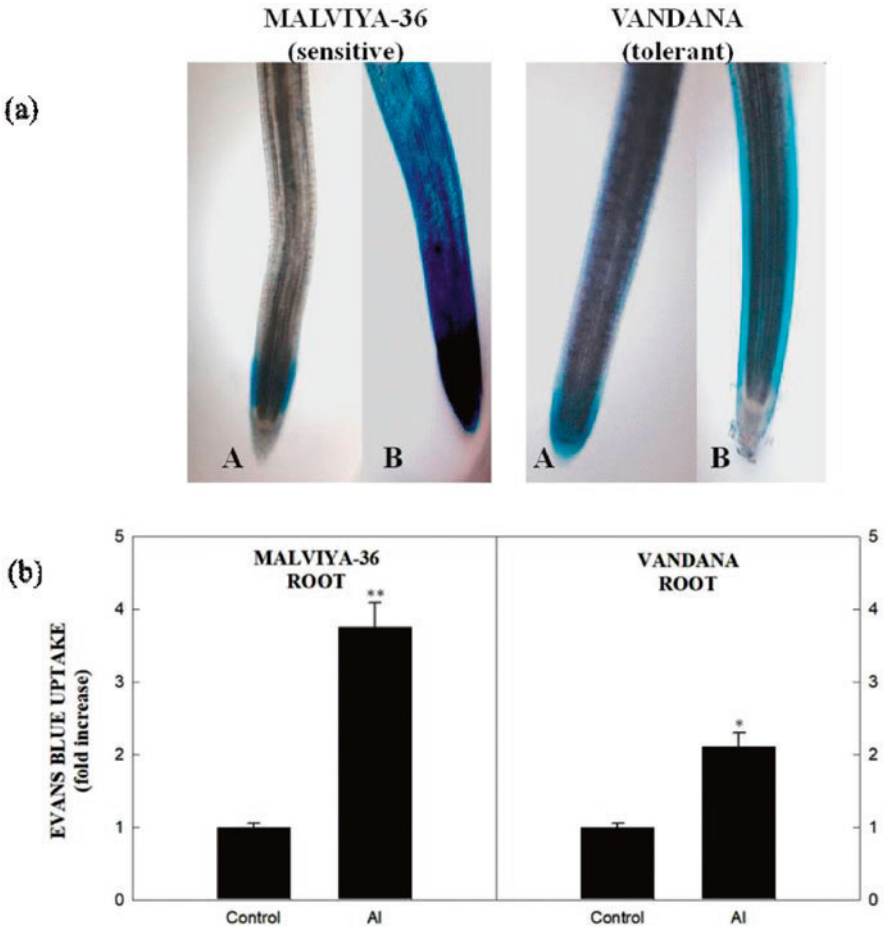

Fig. 4.3 **(a)** Representation of loss of plasma membrane in root tips of Malviya-36 and Vandana seedlings through histochemical detection. The seedlings were grown in sand cultures for 10 days followed by its treatment in either Yoshida nutrient solution (control, without Al^{3+}, denoted as *A*) or in nutrient solutions containing 1mM Al^{3+} (*B*) > for 48 h. **(b)** The loss of plasma membrane integrity after exposure of dye Evans's blue is depicted. The resultant value after three independent values is mean ± SD (standard deviation depicted by bars). As per Turkey's test, * and ** depict the differences as per controls at $p \leq 0.05$ and $p \leq 0.01$, respectively

3.4 *Eriochrome Cyanine R Staining*

When cultivars were kept for 12 days with or without nutrient solution constituting 1mM $AlCl_3$ in the growth medium followed by staining with Eriochrome cyanine R; depicted that the stain taken up by roots of treated seedlings of Malviya-36 was substantially greater than that of Vandana (Fig. 4.4). Control grown seedlings of any cultivar show no stain in their roots.

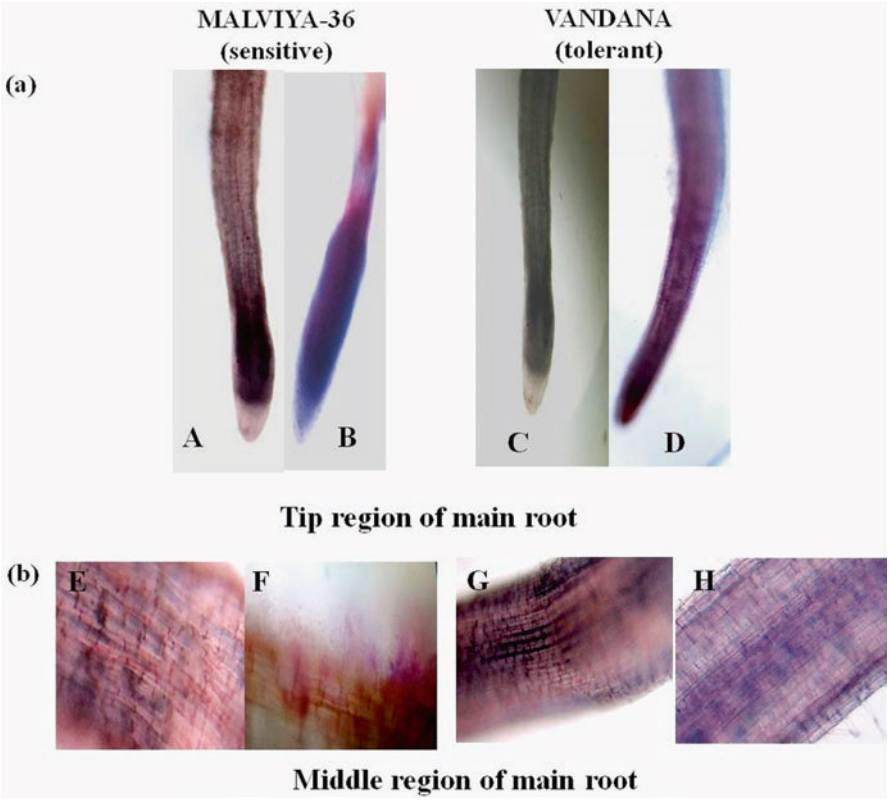

Fig. 4.4 Pictures showing **(a)** tip regions and **(b)** middle regions of main roots after Eriochrome cyanine R staining in Malviya-36 and Vandana seedlings. The seedlings were grown in sand cultures for 10 days followed by its treatment in either Yoshida nutrient solution (control, without Al^{3+}, denoted as *A*, *C*, *E*, *G*) or in nutrient solutions containing 1mM Al^{3+} (*B*, *D*, *F*, *H*). > for 48 h

3.5 Callose Accumulation

The production of callose has been regarded as a measure for determining the level of Al damage to the treated plants. Aniline blue staining and fluorescence microscopy are used to visualize callose formation. Callose exhibited bright green-yellowish spots under the fluorescence microscope, whereas the initial fluorescence observed was vivid blue (Fig. 4.5). The sensitive type root tips that had not been treated fluoresced faintly, and those exposed to Al fluoresced brightly, indicating

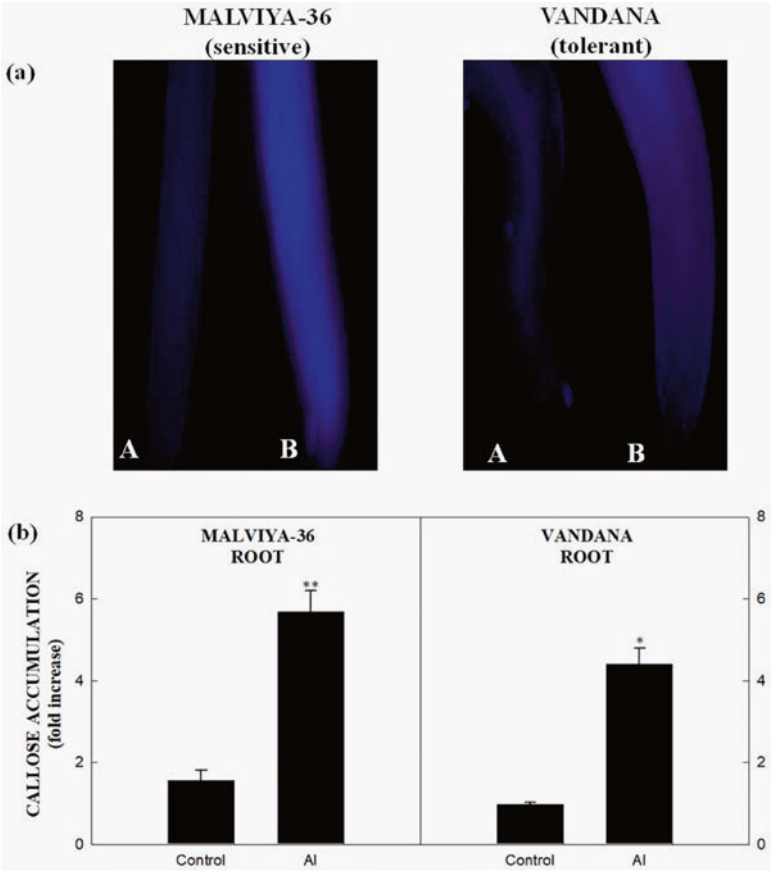

Fig. 4.5 **(a)** The representation of callose localization on exposure of aniline blue staining in root tips of Malviya-36 and Vandana seedlings. Seedlings were grown in sand cultures for 10 days followed by its treatment in either Yoshida nutrient solution (control, without Al^{3+}, denoted as *A*) or nutrient solutions containing 1mM Al^{3+} (*B*) for 48 h. **(b)** The quantification of the root tips grown after control and the seedlings after treatment with Aniline blue stained was recoded. The resultant value after three independent values is mean ± SD (standard deviation depicted by bars). As per Turkey's test, * and ** depict the differences as per controls at $p \leq 0.05$ and $p \leq 0.01$, respectively

callose accumulation. In the presence of Al, callose accumulation in the tolerance cultivar decreased considerably to levels somewhat higher than those reported in the untreated controls, compared to the sensitive variety. When dye uptake for callose accumulation was compared to the control in roots of 1mM, Al^{3+}-treated seedlings, dye uptake for callose accumulation was increased by 5.7- and 4 fold in cv. Malviya-36 and cv. Vandana, respectively, implying callose induction to be an early and robust Al stress indicator.

3.6 Invertase Activity Assay

Tolerant roots (Vandana) exhibited strong activity (cellular divisions in the basal part and more noticeable in the apex) and invertase expression after its exposure to 1mM $AlCl_3$ for 2 days (Fig. 4.6). When compared to tolerant roots (Malviya-36), invertase activity was lower in sensitive roots (Malviya-36) (Fig. 4.6).

4 Discussion

For a long time, aluminum has been regarded as one of the key problems restricting agricultural output around the world. A major portion of the arable land across the world is acidic with significant Al toxicity. A vast area of such land lies in developing countries. Al was, therefore, a potential threat to these countries' development. It is imperative to choose and breed crops for Al resistance to a fast, reliable screening system to discriminate between sensitive and tolerant genotypes. Plants have built up a few techniques to deal with Al toxicity. Organic acid exudation was first detected as helping to protect snap beans against Al toxicity (Miyasaka et al. 1991). It was found that the cultivars that are Al resistant emit almost eightfold citrate than the sensitive ones in a long run. Despite several attempts in past, most of the works could not justify the tolerance of rice to Al toxicity. Another notion of rice resistance was cell wall polysaccharides (Yang et al. 2008). As a result, it's critical to understand its molecular and genetic mechanisms for Al tolerance.

The toxic symptoms of Al^{3+}-treated roots in our research were essentially the same as those previously discovered (Alvarez et al. 2012). Sivaguru and Horst (1998) have reported the distal region to be more Al sensitive in the root apex. As a result, root length is the most reliable metric for determining Al^{3+} toxicity tolerance and sensitivity. In the present study, Al^{3+} inhibited root growth in both rice cultivars, with Malviya-36 being significantly more pronounced than Vandana. The use of a fluorochrome such as Morin (2,3,4,5,7-pentahydroxyflavone), which forms a fluorescent complex with aluminum (Al) (Eticha et al. 2005) and thus used for the detection of Al at the outside of roots with significant differences among cultivars, is the simplest and widely used method for Al localization. At low pH, it is found to be selective to Al and has a detection limit of 2 nM in vitro (Lian et al. 2003). Al^{3+}

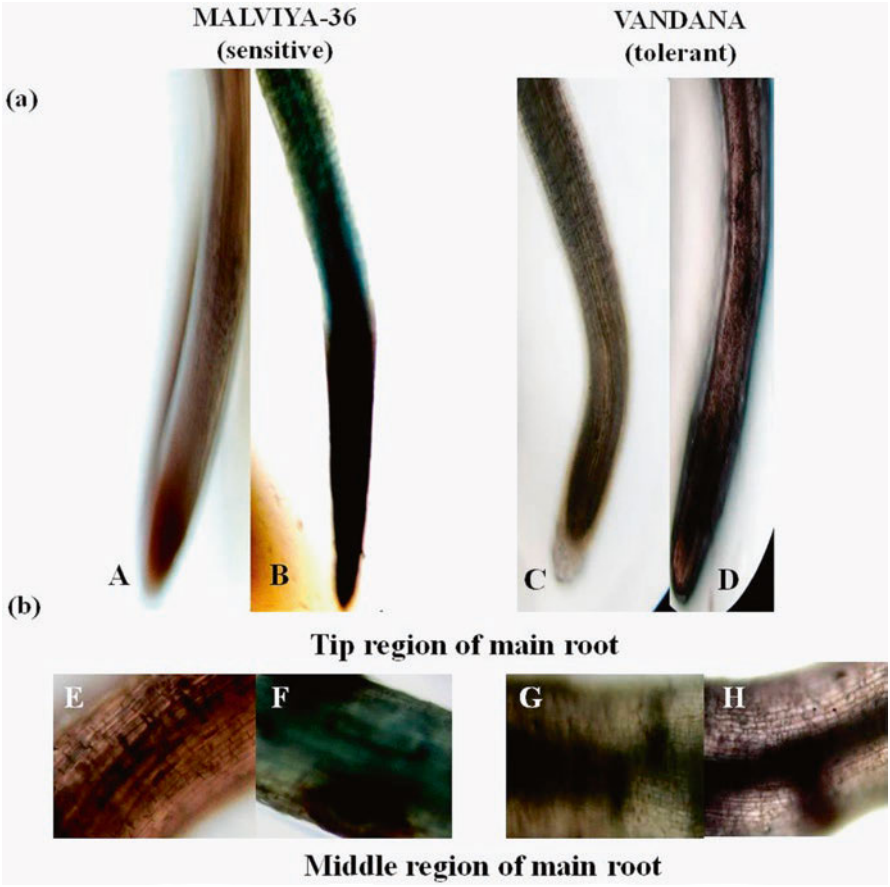

Fig. 4.6 **(a)** Tip regions and **(b)** middle regions of primary roots showing invertase activity staining patterns in Malviya-36 and Vandana seedlings. The seedlings were grown in sand cultures for 10 days followed by its treatment in either Yoshida nutrient solution (control, without Al^{3+}, denoted as *A*, *C*, *E*, *G*) or in nutrient solutions containing 1mM Al^{3+} (*B*, *D*, *F*, *H*). > for 48 h

works as a mordant in hematoxylin staining, binding to oxidized hematoxylin (hematein) and forming a colorful complex between hematoxylin and root-bound Al (Polle et al. 1978). In comparison to cv. Malviya-36, hematoxylin staining revealed decreased uptake of the Al by roots than that of Vandana seedlings. It was also found that Al content is more in the roots of Malviya-36 than that of Vandana. It was also observed that the Al content amplified with rise in the concentration of Al treatment as well as its exposure. Vandana's roots absorb less Al, indicating that this cultivar benefits from an external resistance mechanism. Alike outcomes were conveyed for hematoxylin staining in rice (Pandey et al. 2015; Rosello et al. 2015), wheat (Shao et al. 2015), and chickpea (Sharma et al. 2015). The damage in the Al-induced membrane as observed using Evans's blue could be attributed to prolonged exposure that resulted in mechanical disturbance of cells in the elongation

zone. More exposure of these stains by roots of Al^{3+}-treated saplings of sensitive cv. Malviya-36 suggests more damage and cell death in this cultivar than similarly stressed seedlings of tolerant cv. Vandana. In the roots of cv. Malviya-36, cell death paralleled with high Al-induced ROS production and severe oxidative stress which indicates that cell demise happened because of the damage caused by overproduced ROS in cells by Al^{3+} stress. According to Pan et al. (2001), increased ROS production could result in cell death, and higher Al concentrations could cause root cell necrosis. Even with modest Al toxicity treatment, a considerable increase in Evans blue dye absorption was observed in the prior study (Pandey et al. 2015). Similar findings have been found in maize (Wang et al. 2015), tobacco (Sivaguru et al. 2005), Melaleuca tree (Tahara et al. 2008), pea (Motoda et al. 2011), and wheat (Motoda et al. 2011). (Aggarwala et al. 2015). Evans blue uptake by roots of barley seedlings was also seen by Zelinová et al. (2011), even at low Al concentrations that did not hinder root growth. Al absorption and cell death in root tips are not only a substantial result of Al-induced oxidative stress, but they also serve as one of the Al tolerance mechanisms (Giannakoula et al. 2010). Current work incorporates Eriochrome cyanine R for classifying Al sensitivity and rice cultivar tolerance by showing a much higher stain uptake in roots of Al^{3+}-treated seedlings of cv. Malviya-36 than cv. Vandana. The root section formed following Al treatment was white (unstained) when Al treatment did not harm the root apical meristem, whereas roots impacted by Al treatment were intensely pink stained (Aniol 1995). This method takes only 10 min to stain and was appropriate for assessing large numbers of plants at once. This rapid staining method has been used to screen Al tolerance in wheat, barley, and rye cultivars (Wang et al. 2006; Ma et al. 2004; de Sousa et al. 2016).

Aluminum-induced callose seems to be a physiological marker for Al-induced injury. Callose builds up in the cell wall around plasmodesmata in response to the Al damage within the roots. Increased Al concentration from 0 to 100 mM could result in an enlarged accumulation of callose (Larsen et al. 1996). The lack of callose deposition suggests that there are more primary mechanisms of Al^{3+} resistance that could activate sooner than callose synthesis.

Thus, callose deposition could not be attributed as a primary resource for averting Al^{3+} penetration. Though studies reported (Sivaguru et al. 2000; Wissemeier and Horst 1995) callose to be a cell-to-cell inhibitor and Al-induced callose formation in some cultivars root tips, the current study has found callose formation in newly root tips of higher Al concentration. Increased Al tolerance is linked to lower callose formation and lower oxidative stress in transgenic plants. Callose deposition and accumulation especially in aluminum-sensitive cultivars reflect physiological stress and the degree of cumulative cell damage.

The generation of hexoses is maximized by cell wall and vacuolar invertases, which enhance cell respiration, division, and growth (Koch 2004). When compared to Malviya-36, Vandana roots have more invertase activity, which could indicate alterations in primary metabolism and root cell proliferation, as well as a resistance mechanism.

Past studies revealed that increased sucrose activity in rice could lead to hexose accumulation under Al toxicity. Simon et al. (1994) observed reduced invertase acid activity under Al^{3+}. Similar observations were made where increase invertase activity has been observed under arsenic, Al, and Cd toxicity (Verma and Dubey 2001; Jha and Dubey 2004; Shahnawaz et al. 2017) and salinity and cold stress (Mishra and Dubey 2008; Livingston and Hensen 1998).

The current results demonstrated better and improved discriminating Al susceptibility in variants of cultivars using several staining techniques. Thus, the hypothesis taken has confirmed that high Al concentration results in reduced growth and callose accumulation, associated with invertase activity and cell deaths. It is clear that improvised root growth at toxic Al^{3+} concentrations is accompanied by increased invertase activity, and thus better understanding of expression and activities in root regions could be made at apoplastic and symplastic root regions.

Acknowledgments The first author is obliged to the University Grants Commission (UGC) for providing Fellowship to conduct this work.

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