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Full Length Article

Effects of Aluminum, Iron and/or Low pH on Rice Seedlings Grown in Solution Culture

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Abstract

Water in the paddy field covered by acid sulfate soils having very low pH contains high amount of Al and Fe that affects rice growth. A laboratory study was conducted to qualify rice grown under the adverse conditions can withstand the stresses. Two rice varieties, MR 219 and MR 253, were grown hydroponically at various pH (3, 4, 5, 6, 7), Al (0, 20, 40, 60, 80, 100 µM) and Fe (0, 20, 40, 60, 80, 100 µM) concentrations. After 14 days, rice root length and surface area were determined using a root scanner. Thereafter, organic acids released by the roots of rice were determined by high performance liquid chromatography. Results showed that the root length decreased with increasing Al and/or Fe concentration. On the contrary, the root length increased linearly as the pH of the solution increased. This phenomenon was probably in part related to the exudation of oxalic, citric and malic acids by the rice roots. It was observed that the amount of organic acids released was increased with increasing Al and/or Fe concentration in the solution culture. Hence, it is believed that these organic acids were responsible for chelating some of the Al and/or Fe in the solution, rendering them unavailable for their uptake by rice. In this way, rice plants can withstand some degree of Al^{3+} and/or Fe^{2+} toxicity. © 2015 Friends Science Publishers

Keywords: Acid sulfate soil; Chelation; Aluminum toxicity; Iron toxicity; Organic acid; Rice seedling
Abbreviation: HPLC = High performance liquid chromatography; GML – Ground magnesium limestone; MARDI = Malaysian Agricultural Research Development Institute; SAS = Statistical analysis software; SEM = Scanning electron microscope

Introduction

Rice (*Oryza sativa*) is a staple food for over half of the world's population. More than one billion people depend on rice cultivation for their survival. Realizing the importance of rice and its economic role, the production of rice should be increased sufficiently because growth in rice production has been slower than the population growth. With little room for area expansion, improving the fertility of marginal soils (such as acid sulfate soils) is one of the ways to increase rice production worldwide. Acid sulfate soils occurring along the coastal plains of Peninsular Malaysia (Enio *et al.,* 2011) can be used for rice cultivation if properly ameliorated (Shamshuddin, 2006). Under normal condition, rice growing on these soils performs poorly due Al^{3+}, Fe^{2+} or H^+ stress (Shamshuddin *et al.,* 2013, 2014). It is reported that low pH and high Al and/or Fe concentrations are the result of pyrite (FeS_2) oxidation when the soils are utilized for rice cultivation (Shamshuddin *et al.,* 2004).

At pH <4.8, dissolved Al^{3+}in the soil solution can reach the critical level of 30 µM, which can damage rice plants. Al toxicity is often related to phosphorus deficiency because a soil with high Al concentration will decrease the availability of P due to Al-Fe-phosphate interaction (Ward *et al.,* 2008). That is why the most recognized symptom of Al toxicity is P-related inhibition of root growth.

In acid sulfate soils worldwide, the concentration of Fe^{2+} in the water of rice fields increases when they are flooded for rice cultivation (Muhrizal *et al.,* 2006; Shamshuddin *et al.,* 2014). Toxicity occurs if extractable Fe in the soils exceeds 300 mg kg^{-1} (Dobermann and Fairhurst, 2000). According to Silveira *et al.* (2007), Fe toxicity is considered as one of the most significant constraints in rice production, along with Al toxicity. However, without enough Fe in rice plants, chlorophyll cannot be sufficiently produced (Follett and Westfall, 1992). Under low pH condition, the availability of Fe is increased, causing an excessive accumulation of Fe in the leaves of rice. Visual symptom of Fe toxicity on rice is bronzing (Fairhurst and Witt, 2002). Besides bronzing leaf symptom, Fe toxicity can increase panicle sterility that reduces plant growth (Mathias and Asch, 2005).

According to Elisa *et al.* (2011), Al^{3+} and Fe^{2+} accumulate in rice root due to attraction to the negatively-charged cells wall, preventing cell division and elongation. Rice roots rapidly absorb Al^{3+} and Fe^{2+}, causing a reduction in root length and inhibit root growth that result in the curtailment of nutrient uptake. The end

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result would be the reduction of rice yield (Elisa *et al.,* 2011; Shamshuddin *et al.,* 2014).

Organic acids released by rice roots under stress may play a major role in the Al^{3+} and Fe^{2+} tolerance mechanisms (Gerke *et al.,* 1994; Shamshuddin *et al.,* 2013, 2014) and the utilization of insoluble nutrients, especially P (Jones and Darrah, 1994). There are probably two mechanisms involved in acid metal tolerance by the roots of rice: (1) internal tolerance mechanism in the symplasm; and (2) exclusion mechanism in the apoplasm and at the plasma membrane (Kochian, 1995). For rice, the latter is the more important mechanism than that of the former.

In the exclusion mechanism, metals are prevented from entering or staying in the symplasm or coming into contact with sensitive intracellular sites. For example, Al tolerance is conferred by exclusion from root tip. Elisa *et al.* (2011) found that under stress condition, plant root were found to release greater amount of organic acids into the rhizosphere. These acids chelate Al outside the plasma membrane, thereby reducing its uptake. Ryan *et al.* (2001) suggested that exudation of organic acids could be active mechanism in response to environmental signals and stresses.

Organic acids are known as Al-chelating molecules. A study by Pineros and Kochian (2009) showed that low molecular weight organic acids secreted by plant root chelate Al in the soil solution, forming Al-citrate or Al-malate, preventing it from entering the root cells. Many tolerant plants share this general resistance mechanism. However, the organic acids so released by plant roots vary depending on plant species (Kochian *et al.,* 2005). Miyasaka *et al.* (1991) found that the amount of organic acids secreted by plant root may determine its resistance to Al in the rhizosphere. This finding is in line with that of Ma *et al.* (2001) and Horst *et al.* (2010) who proved that plant root plays a critical role in the tolerance mechanism based on efflux of the organic acids. The most effective organic acid in the alleviating toxic Al and Fe effects was citric, followed by oxalic and tartaric acids. Malic, malonic and salicylic acids were moderate in detoxifying Al and Fe (Yang *et al*., 2013).

According to Malaysian Agricultural Research Development Institute (MARDI, 2010), rice variety MR 219 is a high yielding variety and therefore is widely grown in Peninsular Malaysia. Recently, a new rice variety named MR 253 has been released in Malaysia. This MR 253 rice variety, claimed to be acid tolerant by MARDI, is able to perform better than that of MR 219 in marginal soils. So far, no detailed study has been conducted to determine the actual performance of MR 253 variety on the acid sulfate soils. Hence, a study was conducted to determine the effects of Al^{3+}, Fe^{2+} and low pH on root elongation and root surface area of rice varieties MR 219 and MR 253 and to explore the underlying mechanism of reducing H^+ stress as well as $Al^{3+}$and Fe^{2+} toxicity detoxification.

Materials and Methods

Soils and their Locations of Sampling

Soils and water samples were taken from a paddy field in the Integrated Agricultural Development Project (IADP), Kelantan, which is located in the northeastern part of Peninsular Malaysia. The soils for the characterization and classification purposes were taken at 5 depths: 0–15, 15–30, 30–45, 45–60, and 60–75 cm using an auger.

Soil and Water Analyses

Soil pH (in water) was determined by a pH meter. Exchangeable Ca, Mg, K and cation exchange capacity (CEC) and exchangeable cations were determined using ammonium acetate buffered at pH 7 (Benton, 2001). Exchangeable Al was extracted by 1 M KCl (Barnhisel and Bertsch, 1982) and the Al in the extract was determined by inductively coupled plasma optical emission spectrometry (ICP-OES). Total C and N were determined by CNS TruMac Analyzer. Available P was determined by the method of Bray and Kurtz (1945) with the extracted P determined by auto analyzer (AA). Extractable Fe in the soils was determined by double acid method. It was extracted using 0.05 M HCl in 0.0125 M H_2SO_4. A 5 g sample of the soil was mixed with 25 mL of the extracting solution and shaken for 15 minutes. The solution was then filtered through Whatman filter paper number 42 before determining the Fe it contained by ICP-OES. The water taken from the paddy field was filtered through Whatman filter paper number 42 before determining its pH. The concentration of Al and Fe in the water was determined by ICP-OES.

Plant Material

Rice (*Oryza sativa* L.) varieties MR 219 and MR 253 used for this study were taken from Department of Agriculture, Pasir Mas, Kelantan, Malaysia.

Location, Materials and Experimental

This study was carried out at the Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Malaysia. It was conducted in a laboratory under hydroponic condition at the ambient temperature. Seeds of rice (varieties MR 219 and MR 253) were soaked in a hormone-based chemical (Zappa™) for 24 h and then left in the dark place for another 24 h.

This was a short-term experiment conducted in two phases; the method used was modified from that of Elisa *et al.* (2011). In the first phase experiment, three pre-soaked seeds were transferred into test tubes containing 0.5 mM $CaCl_2$ solution at various concentrations of Al (0, 20, 40, 60, 80 and 100 μM) using $AlCl_3$, Fe (0, 20, 40, 60, 80 and 100 μM) using $FeCl_3$ and mixtures of Fe and Al (0, 20, 40, 60, 80 and 100 μM). In the second phase experiment, three pre-soaked seeds were transferred into test tubes containing water taken from the paddy field. For this experiment, the pH of the water was adjusted to various levels (3, 4, 5, 6 and 7) using either 0.01 M HCl or 0.01 M NaOH. For both phases of the study, the seeds of rice were allowed to grow in the tubes for 14 days. No fertilizers were added to promote the growth of the rice seedlings.

Determination of Root Morphology

At day 14, root length (cm) and root surface area (cm^2) of rice for both phases of the study were determined using a root scanner, model Win RHIZO 2012.

Visual Observation of Rice Root

Rice root was observed under scanning electron microscope (SEM). The roots of the rice seedlings were cut into pieces, pre-fixed with 4% glutaraldehyde overnight and washed with 0.1 M sodium cacodylate buffer 3 times for 30 minutes. Osmium tetraoxide buffer (1%) was used for post fixation. After series of dehydration in acetone (35, 50, 75, 95 and 100%), the samples were dried in a critical point dryer and mounted on aluminum stubs, sputter-coated in gold and viewed under SEM (JEOL JSM-6400 attached with OXFORD INCA ENERGY 200 EDX).

Determination of Organic Acids

The organic acids in the culture solution for both phases of the study were determined using a high performance liquid chromatography (HPLC), Jasco Borwin software. About 20 µL of the samples from each treatment were injected into the HPLC with a UV detector set at 210 ηm, using a Rezex ROA-organic acid H^+ (8%) column (250 × 4.6 mm) from Phenomenex Co.; 0.005 N H_2SO_4 was the mobile phase with a flow rate of 0.17 mL min^{-1}. Peaks for the organic acids detected were identified by comparing with the retention times obtained for pure organic acids injected as standards. From the peak areas, the quantity of organic acids in the samples were calculated and expressed in µM.

Determination of Sensitivity to Tolerance

Two rice varieties were studied to differentiate their sensitivity to Al, Fe and low pH stress. It was done via 2 methods: (1) using the slope of the graphs of the relative root length or root surface area against Al or Fe concentration; and (2) using the critical values for rice growth. The steepness of the slope of the graphs and critical values (based on the 90% relative root length or root surface area of rice seedlings) were used as a measure of Al or Fe tolerance.

Statistical Analysis

The experiments were laid out in Completely Randomized Design (CRD) with four replications. The data collected from this study were analyzed by ANOVA for analysis of variances and Tukey test for mean comparison using SAS version 9.2 (SAS Institute, Inc., Cary, N.C., USA). All diagrams in this paper were drawn using Excel Program in the Microsoft.

Results

Initial Chemical Properties of Soil and Water

Table 1 shows the chemical characteristics of the acid sulfate soil under study. The pH of the topsoil was 3.64. Soil pH decreased consistently with depth, the lowest being 3.13 at the 60–75 cm depth. The exchangeable K, Ca and Mg in the topsoil were below the sufficiency level for rice growth, while the exchangeable Al in the same horizon was very high (> 5 $cmol_c$ kg^{-1}). The available P was also low (< 5 mg kg^{-1}) in the topsoil. Table 2 shows that water pH was 2.98, while the concentration of Al and Fe were 1060 and 192 µM, respectively.

First Phase Experiment

Effect of Al on the root length and root surface area: The relative root length of MR 219 and MR 253 rice varieties were negatively and highly correlated with Al concentration and the equations representing the relationship are given by Y = 103.54 - 0.89x (R^2 = 0.97) and Y =107.45 - 1.07x (R^2 = 0.94), respectively (Fig. 1a). The relative root surface area of rice seedlings for both varieties showed the same trend. They are negatively and highly correlated with Al concentration and the equations representing the relationships are given by Y = 97.13 - 0.80x (R^2 = 0.95) and Y = 101.32 - 0.97x (R^2 = 0.97), respectively (Fig. 2a). Fig. 2a shows that the slope of the graph for rice variety MR 253 was steeper compared to that of MR 219, while the critical Al concentration for variety MR 219 and MR 253 were 8.91 and 11.67 µM, respectively.

Effect of Fe on the root length and root surface area: The relative root length of MR 219 and MR 253 was negatively correlated with Fe concentration and the equations representing the relationship are given by Y = 106.57 - 0.92x (R^2 = 0.98) and Y = 103.84 - 0.60x (R^2 = 0.98), respectively (Fig. 1b). The relative root surface area for both varieties showed similar trend. The equations representing the relationships are given by Y = 101.68 - 0.62x (R^2 = 0.93) and Y =97.01 - 0.80x (R^2 = 0.98), respectively (Fig. 2b). The slope of the graph for MR 219 was less steep compared to that of MR 253. The critical Fe concentration for the growth of varieties MR 219 and MR 253 were 18.84 and 8.76 µM, respectively. On the contrary, MR 253 was more tolerant to Fe compared to that of MR 219 if it is based on the root length (Fig. 1b).

Table 1: The chemical properties of the acid sulfate soil used in the study

Depth	pH	EC	Exchangable cations ($cmol_c kg^{-1}$)				Ext. Fe	*CEC	Total N	Avail. P	Total C
(cm)		mS/cm	Ca	Mg	K	Al	($mg\ kg^{-1}$)	($cmol_c kg^{-1}$)	%	($mg\ kg^{-1}$)	%
0-15	3.64	0.69	0.02	0.15	0.04	5.53	135.4	15.57	0.003	4.72	4.90
15-30	3.51	0.57	0.05	0.10	0.02	6.43	208.5	11.12	0.004	4.28	1.05
30-45	3.25	0.59	0.05	0.25	0.01	6.04	286.0	6.52	0.003	5.38	0.97
45-60	3.24	0.59	0.04	0.26	0.03	5.82	483.1	9.58	0.003	40.46	1.26
60-75	3.13	0.63	0.03	0.29	0.03	6.15	368.2	6.99	0.002	41.09	2.41

*CEC = cation exchange capacity

Table 2: The chemical properties of the water taken in paddy field at acid sulfate soil area

pH	EC	Basic cations (µM)			Fe	Al	P
	mS/cm	Ca	Mg	K	(µM)	(µM)	($mg\ L^{-1}$)
2.98	0.65	35.75	50.83	33.33	192	1060	29.03

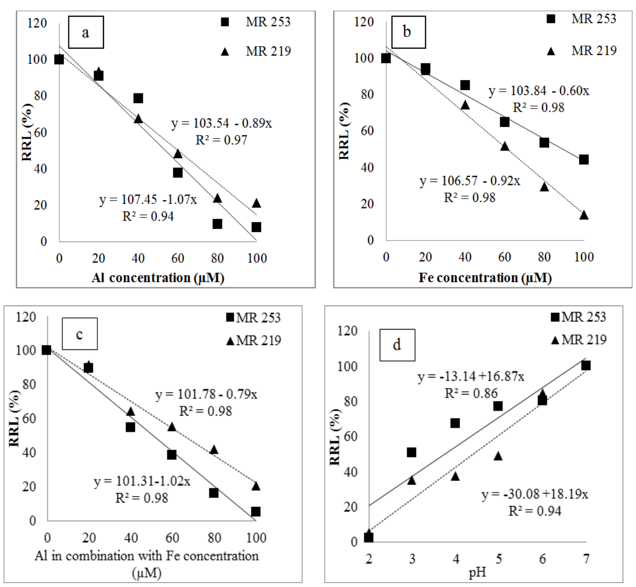

Fig. 1: Relationship between relative root length (RRL) and a) Al concentration, b) Fe concentration, c) Al in combination with Fe concentration and d) pHofMR 219 and MR 253

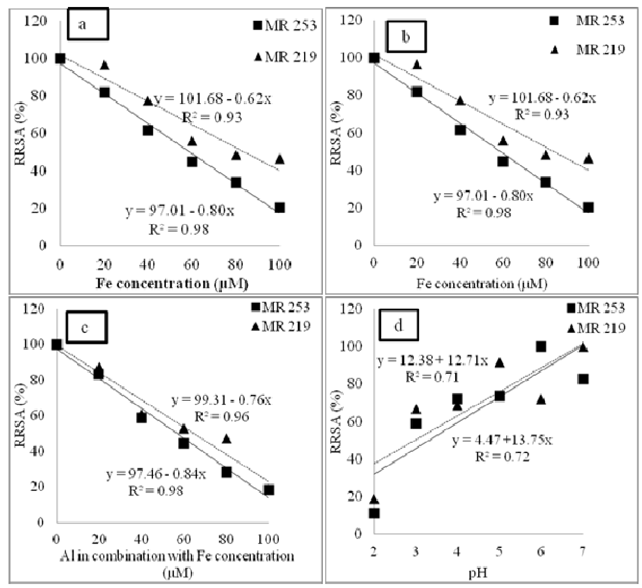

Fig. 2: Relationship between relative surface areas (RRSA) and a) Al concentration, b) Fe concentration, c) Al in combination with Fe concentration and d) pH of MR 219 and MR 253

Effect of Al in combination with Fe on the root length and root surface area: The relative root length of MR 219 and MR 253 was negatively correlated with Al in combination with Fe and the equations representing the relationship are given by Y =101.78 - 0.79x (R^2 = 0.98) and Y = 101.31 - 1.02x (R^2 = 0.98), respectively (Fig. 1c). The relative root surface area for both rice varieties showed the same trend. The equations representing the relationships are given by Y = 99.31 - 0.76x (R^2 = 0.96) and Y = 97.46 - 0.84x (R^2 = 0.98), respectively (Fig. 2c). The slope of the graph for MR 219 was less steep compared to the slope of MR 253. This means that MR 253 was more sensitive to Al in combination with Fe compared to that of MR 219. The critical Al in combination with Fe for variety MR 219 and MR 253 were 12.25 and 8.88 µM, respectively.

Second Phase Experiment

The relative root length of MR 219 and MR 253 was positively correlated with pH and the equations representing the relationship are given by Y = -30.08 +18.19x (R^2 = 0.94) and Y = -13.14 + 16.87 x (R^2 = 0.86), respectively (Fig. 1d). The relative root surface area of rice seedlings for both varieties showed similar trend. The equations representing the relationships are given by Y =12.38 + 12.71x (R^2 = 0.71) and Y =4.47 + 13.75 x (R^2 = 0.72), respectively (Fig. 2d). In Fig. 2d, it is shown that the critical pH values for rice varieties MR 219 and MR 253 growth are 6.1 and 6.2, respectively. This means that MR 219 and MR 253 were more or less comparable in terms of their tolerance to low pH stress.

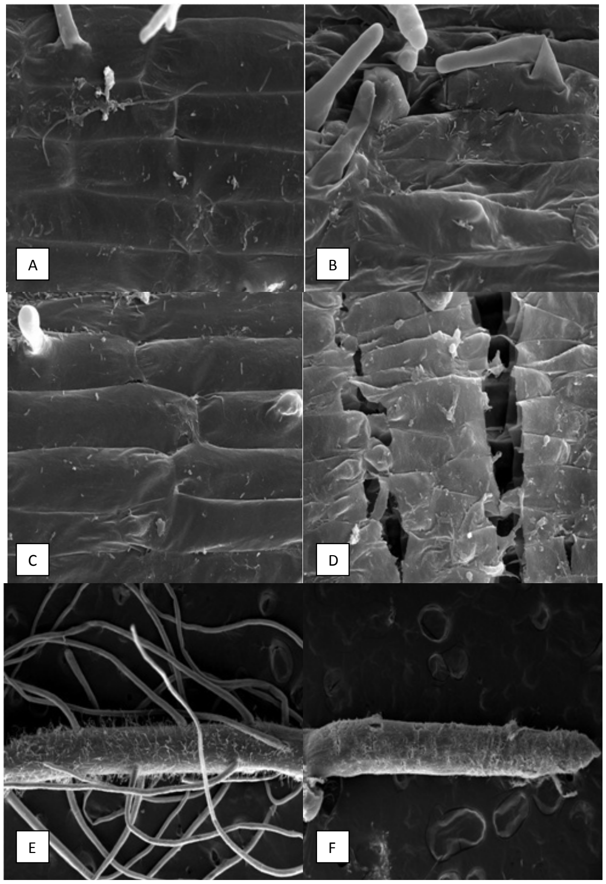

Fig. 3: Root surface of rice seedlings viewed using SEM after 14 days treatment. A) MR 219 at 0 µM of Fe + Al showing smooth surface. B) MR 219 root surface at 100 µM of Fe + Al; note shrinking surface due to Al and Fe stress. C) MR 253 at 0 µM of Fe + Al showing smooth surface. D) MR 253 root surface at 100 µM of Fe +Al; note torn surface tissue Al and Fe stress. E) Root surface at pH 7 acid sulfate soil solution showing; note root hairs developed very well. F) Root surface under low pH

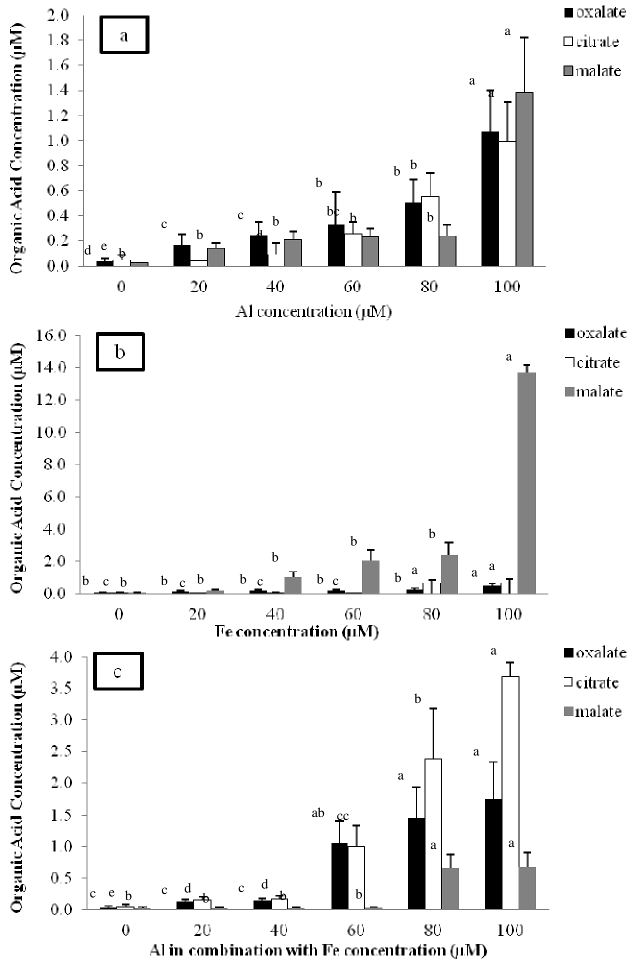

Fig. 4: Exudation of organic acids by rice root MR 219 under a) Al concentration b) Fe concentration c) Al in combination with Fe concentration

SEM observations on the cellular structures of the roots showed that both rice varieties were affected by the high concentration of Al in combination with Fe. Fig. 3 shows that root cellular structures of MR 219 shrank due to the presence of Al plus Fe at 100 µM, while those of MR 253 were ruptured. The roots of the rice seedlings were also affected by the low pH as shown in Fig. 3F. For rice growing under high pH condition, its root hairs were well developed; however, when the pH was reduced, the root hairs were either absent or their development was significantly curtailed.

Secretion of Organic Acids by Rice Root under Al Stress

Fig. 4a and 5a show organic acids exuded by MR 253 was higher compared to those of MR 219. Malate exuded by MR 253 was probably activated by the exposure to Al and was significantly higher compared to that of MR 219. However, there is no significant difference in citrate and oxalate exudation between both varieties at low Al concentration. Fig. 4a shows that the root of MR 219 secreted high amount of organic acids at high Al concentration. The exudation of organic acids by MR 253 showed the same trend as that of MR 219 (Fig. 5a).

Organic Acids Secretion under Fe Stress

As depicted in Fig. 4b and 5b, both rice varieties secreted high amount of organic acids at high concentration of Fe, with MR 253 secreted more organic acids compared to that of MR 219, especially citrate and oxalate. For MR 219, the root secreted higher amount of malate compared to that of MR 253.

Organic Acids Secretion under the Stress of Al in Combination with Fe

It is seen in Fig. 4c and 5c that organic acids exuded by MR

253 was higher than that of MR 219. Malate, citrate and oxalate exuded by MR 253 was activated by the exposure to Al and Fe and were significantly higher compared to that of MR 219. The root of MR 219 secreted more organic acids at high Al and Fe concentration compared to those at low concentration (Fig. 4c). As the Al and Fe increased, the exudation of organic acids was increased. The exudation of organic acids by MR 253 showed the same trend as that of MR 219 (Fig. 5c).

Organic Acids Secretion under Low pH Stress

The exudation of organic acids due to H^+ stress for variety MR 219 and MR 253 is depicted in Fig. 6 and Fig. 7, respectively. It was observed that the root of MR 219 secreted higher amount of organic acids at low pH compared to those at high pH. As the pH increased, the exudation of organic acids was reduced. The trend for the exudation of organic acids by MR 253 was similar to that of MR 219 (Fig. 7).

Discussion

The soil under study was a true acid sulfate soil. It was classified as Typic Sulfaquept (Soil Survey Staff, 2010), shown by its low soil pH (< 3.5) and the presence of yellowish jarosite within the 0–50 cm depth (Shamshuddin, 2006). Under this condition, the concentration of Al and Fe in the water was expected to be above the critical level for rice growth (Shamshuddin *et al.,* 2013, 2014). The water pH was also expected to be below the critical level of 6 (Elisa *et al.* 2011).

The concentration of Al in water is dependent on its pH. As the pH falls below 5.0, the concentration of Al increases. In the current study, the pH of the water was 3.98; hence, Al concentration was expected to be high, exceeding the critical level for rice growth (Shamshuddin *et al.,* 2013, 2014). Al concentration in the water of 1060 µM was far above the critical level of 15 µM (Elisa *et al.,* 2011). Data in Table 1 show that the exchangeable Ca and Mg were below the critical level. The required level of Ca is 2 $cmol_c\ kg^{-1}$ soil (Palhares, 2000), while that of Mg is 1 $cmol_c kg^{-1}$ soil (Dobermann and Fairhurst, 2000). Ca and Mg deficiencies can be alleviated by applying ground magnesium limestone (GML) at the appropriate rate (Shamshuddin, 2006, 2014).

Due to high Al concentration, the growth of rice root was inhibited. Many studies have been conducted to explain the causes of Al inhibiting the growth of crop roots. For instance, Liao *et al.* (2006) found that non-tolerant Al plants were badly affected by exposure to Al. Al disturbs the growth of cells in the elongation zone of roots (Bian *et al.,* 2013). Furthermore, Barker and Pilbeam (2007) explained that Al can reduce root cell division; hence, it causes disruption of root cap processes, inhibiting root elongation. We believed that Al can affect rice roots in similar fashion. Elisa *et al.* (2011) explained that soluble Al^{3+} accumulated in the rice root tissue prevented cell division and eventually its elongation. It is likely that rice variety MR 253 was more tolerant to Al compared to that of MR 219 (based on root surface area). However, data from root length study showed otherwise. It probably means that these two rice varieties have no difference in terms of their tolerance to Al toxicity.

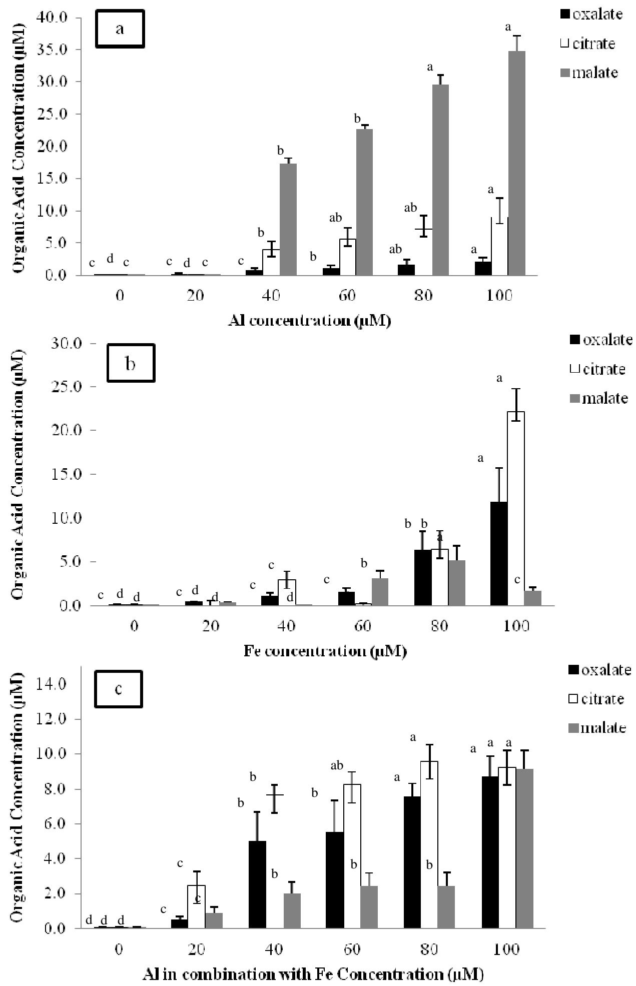

Fig. 5: Exudation of organic acids by rice root MR 253 under a) Al concentration b) Fe concentration c) Al in combination with Fe concentration

We believed that more Al^{3+} were accumulated in the root apex (elongation zone, root cap and meristem) compared to that of the mature root tissue. Initially, the root apex rapidly bound Al^{3+} in the apoplast with the rate becoming lower thereafter (Bian *et al.,* 2013). The rapid binding of Al was probably due to the presence of pectic matrix in the apoplast cell walls. This pectic matrix contained carboxylic groups that were negatively-charged and have high affinity for Al^{3+} (Chang *et al.,* 1999). The binding between the pectic matrix and Al caused loosening of enzyme in the cell walls, resulting in the loss of its function. Hence, it decreased cell wall extensibility physically and physiologically. Thus, the root length was

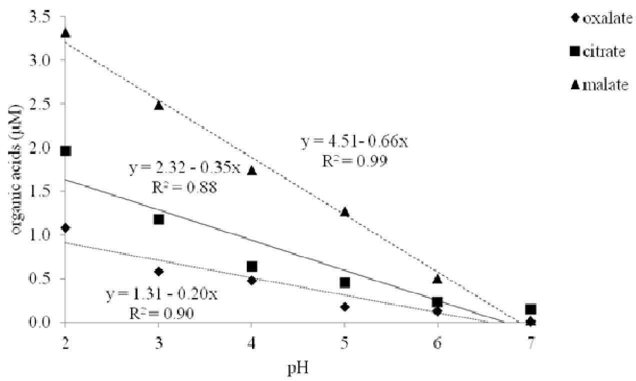

Fig. 6: Exudation of organic acids by rice root MR 219 under pH stress

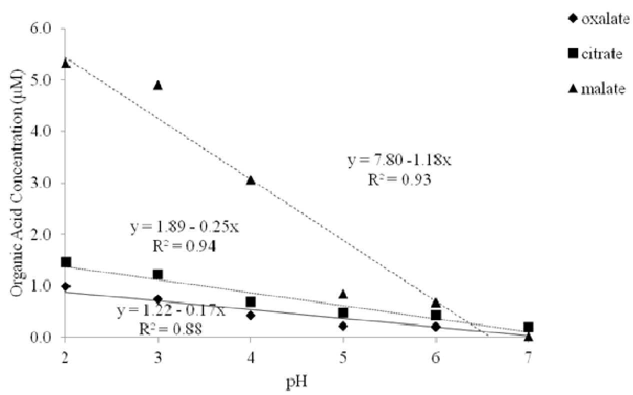

Fig. 7: Exudation of organic acids by rice root MR 253 under pH stress

reduced and the root growth was curtailed due to less nutrients uptake (Wehr *et al.,* 2004).

Plasma membrane can acts as a barrier of Al because ions like polyvalent Al^{3+} are insoluble in the lipid bilayers. However, Klug *et al.* (2011) found that up to one half of the total of Al in the root apex was located in the symplasm. When Al crossed the membrane, many potential harmful interactions can occur. Al bound to the lipid bilayer inhibited transport, displaced ions from critical sites and disrupted intracellular metabolism (Delhaize and Ryan, 1995).

The critical level for Fe varies depending on the vegetative stage of rice growth. According to Dobermann and Fairhurst (2000), highly tolerant rice variety can withstand Fe of up to 300 mg kg^{-1}. In acid sulfate soils, extractable Fe varies widely depending on the environmental conditions. Fe^{2+} (the form of iron toxic to rice) concentration in water increases with flooding and it may exceed the critical level. For this study, the soil was taken during dry season and the extractable Fe in the topsoil was less than 300 mg kg^{-1}, but at the lower depth it exceeded this value. Fe concentration in the water was 192 µM, way above the respective critical level of 18.84 and 8.76 µM found for MR219 and MR 253. Hence, rice planted on this soil is expected to suffer from Al^{3+} and/or Fe^{2+} stress (Zhu *et al.,* 2009; Shamshuddin *et al.,* 2013, 2014).

Root growth was also inhibited by the excess of Fe. The pKa of Fe is 3, so its availability in the water was very high. This metal can be taken up by rice roots in the form of Fe^{2+}. This form of Fe would be available when the paddy field was flooded for rice cultivation (Muhrizal *et al.,* 2006). Plants have two distinct mechanisms to take Fe, Strategy I and Strategy II (Marschner, 1995). Strategy I involves acidification, reduction and transportation at the plasma membrane of the root epidermal cells, while Strategy II is secretion of phytosiderophores in response to Fe deficiency. For rice, it can adapt both mechanisms because it is growing under anaerobic conditions where soluble iron is prevalent. Fe^{2+} is taken up at the root cortex, which enter the xylem. When Fe^{2+} was abundant within the cell, it might catalyze the active oxygen generation (Marschner, 1995). The activity of phenol oxidases increased and the oxidized polyphenols later accumulated, resulting in Fe toxicity (Mathias and Asch, 2005).

It is claimed that MR 253 is slightly tolerant to acidity. However, our results indicated no significant difference between MR 253 and MR 219. We found that at very low pH, the root hairs of both varieties were either absent or poorly developed, but when the pH was above 6, the root hairs were in perfect order. This was because rice requires the pH of about 6 to grow optimally. But, water pH in our study area was 3.98. It means that under natural conditions, acid sulfate soils in Malaysia are unsuitable for rice cultivation.

The critical water pH for both rice varieties was about 6, which is similar to that obtained by Elisa *et al.* (2011). This pH level is rather unusual for the areas covered by acid sulfate soils in Malaysia (Shamshuddin, 2006). To raise water pH to the required level we need at least to apply 4 t GML ha^{-1} (Rosilawati *et al.,* 2014). Even at this rate of lime application, water pH was below 5, meaning that the rice was still subjected to Al toxicity (Shamshuddin *et al.,* 2013, 2014). Liming at higher rate is uneconomical and therefore not sustainable in the long run. However, due to GML application at 4 t ha^{-1} rice is able to grow and produces reasonable yield due to its ability to protect itself against acidity (Shamshuddin *et al.,* 2014).

The pH of water is dependent on Al and/or Fe concentration; the respective pKa of these metals is 5 and 3. At pH below their pKa, the metals dissolved readily to exist in their ionic forms. In the current study, the pH of the water taken from the paddy field was 2.98, while the concentration of Al and Fe were 1060 and 192 µM, respectively. Thus, it was possible that the organic acids so exuded by the rice roots at low pH was not due to H^+ stress only, but more importantly because of the presence of high concentration of Al and/or Fe. This explanation is consistent with that of Pineros and Kochian (2009) who explained that less organic acids were secreted by the rice roots at high pH due to the immobilization of the phytotoxic Al^{3+}. If the organic acids were indeed exuded due to low pH stress as it happens in corn, the plausible mechanism would be as follows. We know that the pKa of many organic acids ranges from 4 to 5 (De Coninck, 1978). In the case of corn, oxalic acid hydrolyzes following this equation:

$$H_2C_2O_4 + H_2O \rightarrow HC_2O_4^- + H_3O^+, \text{pKa} > 4$$

At low pH (for example 3), oxalic acid was exuded by the rice roots. If the water pH was below its pKa value, the reaction would be in the reverse order. That means at the solution-root interface, solution pH would approach the value of 4. Hence, the stress on rice due to low pH condition was somewhat alleviated.

Citric, oxalic and malic acids were released by MR 219 and MR 253 under stress. According to Yang *et al.* (2013), the efficacy of organic acids in the alleviating Al and Fe toxicity was in the order of citric > oxalic > malic. This finding is consistent with the study of Bian *et al.* (2013) and Miyasaka *et al.* (1991) who found that aluminum-tolerant rice cultivar released more citrate than oxalate. The secretion of organic acids could have occurred immediately after the positively-charged Al^{3+} ions touched the negatively-charged membrane surfaces and pectin cell walls (Horst *et al.,* 2010). Once this happened, the organic acids so released from the root cells bound the Al^{3+} to form organo-Al-complex, preventing it from accumulating in the apoplast that could damage the root cells. Hence, the Al^{3+} ions were deactivated and no longer toxic to the rice seedlings.

In the current study, we found that organic acids exuded by MR 253 was higher than that of MR 219, which means that MR 253 was able to reduce more Fe toxicity, making the seedlings grow better compared to that of MR 219. However, this has not been the case. We observed that the root length and root surface area of MR 219 were higher and had higher critical Fe value compared to that of MR 253 even though the secretion of organic acids for MR 219 showed otherwise.

In conclusion, both MR 219 and MR 253 rice varieties were affected significantly by low pH and high Al and/or Fe concentration, shown by the decrease in root length and root surface area with increasing Al and/or Fe concentration. At very high Al and/or Fe concentration, the cellular structures of the rice roots were severely damaged. The respective critical Al concentration for MR 219 and MR 253 rice varieties was 8.91 and 11.67 µM, while that of Fe was 18.84 and 8.76 µM. On the contrary, the root length increased as the pH of the water increased. The best pH for the growth of both rice varieties was about 6. Under adverse conditions, rice has mechanisms to protect itself against Al and/or Fe toxicity. When the concentration of Al and/or Fe was high its roots secreted organic acids that chelated Al and Fe, rendering them unavailable for the uptake by the rice roots.

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