Australian Journal of Crop Science AJCS

AJCS 7(3):314-320 (2013) ISSN:1835-2707

Rice defense mechanisms against the presence of excess amount of Al^{3+} and Fe^{2+} in the water

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Abstract

Rice grown on an acid sulfate soil is subjected to Al^{3+} and Fe^{2+} toxicity. The stress encountered by the rice plant is usually alleviated by applying ground magnesium limestone (GML). A study was conducted to explain how rice planted on acid sulfate soils can overcome the stress caused by Al^{3+} and Fe^{2+} toxicity. The rice variety tested in this study was MR 219. Seed (under H^+ and/or Al^{3+} stress) germination experiments were conducted in the laboratory in which root length, root surface area and organic acids excretion were determined. This study was followed by a liming trial in the field. The results from the laboratory experiments showed that high Al concentration in the water severely affected root length of rice seedlings and caused the release of organic acids by rice roots. Field trial results showed that when GML was applied under flooded condition at the rate of 4 t ha^{-1}, water pH increased from 3 to 4.5. The pKa of Al was 5 and Al concentration was still high in the water, which was most probably existed in the form of Al^{3+}. Although under stress, the rice was able to grow and consequently produced a reasonable yield. This was probably due to excretion of citric, oxalic and malic acids when Al^{3+} was on the surface of the roots, which subsequently chelated the Al^{3+}, thus enabling the rice to defend itself against Al^{3+} toxicity. Rice defends itself against Fe^{2+} via another mechanism. The pKa of Fe is 3. Due to liming at 4 t GML ha $^{-1}$, the pH of the water increased to pH above 3. The Fe was precipitated as brown crust, coating the surface of the rice roots, and subsequently prevented or reduced the uptake of Fe^{2+}, thus the overall effect of Fe^{2+} toxicity on rice plant was less severe. To grow rice on acid sulfate soils, it is recommended that GML should be applied at the rate so that water pH is increased to above 5 to get rid of Al^{3+} and Fe^{2+} although it might be costly.

Keywords: Acid sulfate soil, aluminum toxicity, ground magnesium limestone, iron toxicity, rice.

Abbreviation: AAS = Atomic absorption spectrometry, Al = Aluminum, Ca = Calcium, CEC = Cation exchange capacity, Fe = Iron, GML= Ground magnesium limestone, HPLC = High performance liquid chromatography, HSD = Honestly significant difference, LSD = Least significance difference, Mg = Magnesium, SAS = Statistical analysis software and TE = Trace element.

Introduction

Rice is the most important staple food crop in the developing world, with more than 1 billion people depends on rice cultivation for their livelihoods. It is believed that rice production can be increased by expanding the area cropped to rice or by improving the productivity. With no expansion in area under production in sight and the slowing down in yield increase, the growth in rice production has fallen below demand, and this is exacerbated by population increase. One of the soils targeted for rice cultivation in tropical area right now are acid sulfate soils which are abundant in the region, occurring almost exclusively in the coastal plains (Enio et al., 2011; Shamshuddin and Auxtero, 1991). These soils are characterized by pyrite (FeS_2) which produces high acidity (soil pH< 3.5) when they are exposed to the atmosphere due to drainage, resulting in the release of high amounts of Al^{3+} and Fe^{2+} into the environment (Shamshuddin et al., 2004a), affecting oil palm growth (Auxtero and Shamshuddin, 1991), cocoa production (Shamshuddin et al., 2004b) and rice growth (Elisa et al., 2011). It is known that Al^{3+} is attracted to the roots due to their negatively-charged cell walls, causing toxicity (Yang et al., 2009). Many plants can partially overcome the phytotoxic effects of Al^{3+} by releasing Al-chelating molecules, such as low molecular weight organic acids from the root system which mobilizes Al^{3+} at the root surfaces, preventing it from entering root cells (Pineros and Kochian, 2009). Unless properly ameliorated using appropriate amendments, acid sulfate soils cannot be productively utilized for agriculture. Among the agronomic problems common to acid sulfate soil are Al^{3+} and Fe^{2+} toxicity. In Malaysia, paddy fields are usually irrigated for rice cultivation. On flooding, reduction process takes place slowly, consuming protons along its way (Muhrizal et al., 2006; Konsten et al., 1994). The Fe^{2+} is produced with the amount dependent on the rate of reduction that occurs. The water pH would eventually increases resulting in the precipitation of Fe hydroxides. Meanwhile, the released Fe^{2+} can be taken up by rice roots, thereby causing toxicity. Moormann and van Breemen (1978) believed that rice has a mechanism to reduce the uptake of Fe^{2+}. According to them, rice plant pumped in O_2 downwards through the roots, creating an oxidized zone around it. Ferric hydroxides would precipitate as brown crust, preventing the uptake of Fe^{2+}. However, this mechanism is hard to prove experimentally and therefore cannot be used to explain the reduction of Fe^{2+} convincingly. Among the methods used to ameliorate acid sulfate soil infertility are liming using ground magnesium limestone, applying ground basalt or submerging the soils continuously. Liming is a common approach to raise pH of acidic soils so as to precipitate aluminum as inert Al-hydroxides, thereby reducing its availability to the growing crops (Shamshuddin et al., 2010; Shamshuddin et al., 1991). Besides increasing pH, GML can supply Ca and Mg, which are needed by crops in large amount. To some extent, Ca itself is able to detoxify Al^{3+} (Alva et al., 1986). Likewise, ground basalt application can ameliorate acid soil infertility through pH increase and Ca release (Shamshuddin and Kapok, 2010; Anda et al., 2009). The objective of the present study was to obtain experimental data to show and explain how rice, variety MR 219, was able to grow on acid sulfate soils in the presence of high concentration of Al^{3+} and Fe^{2+}.

Results

Laboratory experiment

Effects of Al and pH on root length of rice seedlings

The pH of water taken in the field at Merbok, Kedah, and used for the laboratory experiment was 3.7 and the Al concentration was 878 µM. This water pH was too low, while the Al concentration in the water was far too high for the healthy growth of rice. This water was used for the seed germination experiment in this study. The result showed that the root length of rice seedlings was affected severely by the presence of high Al concentration in the water. This result is consistent with the finding of Horst et al. (2009) who established that Al^{3+} inhibited plant root elongation. Reduction in root length could have resulted in the decrease of nutrient uptake. The root length of rice seedlings was affected by low pH. Rice root length was positively correlated with pH (Elisa et al., 2011). The rice seedlings grew better under the condition of high pH than that of low pH. The pH of water in the area covered by acid sulfate soils in Peninsular Malaysia is usually less than 4. Elisa et al. (2011) established that the critical Al concentration of the growth of MR 219 was 15 µM. Hence, rice variety MR 219 is less tolerant to Al toxicity than that reported by Dent (1986). Under normal circumstances, Al concentration in the water of the paddy field on acid sulfate soils cropped to rice (even after lime is applied) is above that value. According to Elisa et al. (2011), the critical pH for rice growth is 6.

Exudation of organic acids by rice roots

Roots of rice seedlings were found to release organic acids in the presence of high amount of Al in the water (data not shown). However, in this experiment the amount of oxalic acid released by the seedlings in the three treatments was not significantly different among the treatments. Rice seedlings grown under the condition of Al concentration of 50 µM had released about 0.130, 0.081 and 0.077 µM, oxalic, citric and malic acids, respectively. We know that in the field where acid sulfate soils occur, the water usually contains more than 50 µM Al. Rice roots release more organic acids at low pH than that at high pH (Horst et al., 2009). At the pH 3, the seedlings released oxalate, citrate and malate at 0.009, 0.007 and 0.058 µM, respectively (Elisa et al., 2011). This phenomenon can be explained by higher concentration of Al at low pH; at high pH, Al precipitates as Al-hydroxides (Shamshuddin and Anda, 2012). As the pH increased, less organic acids were secreted by the rice roots due to the immobilization of the phytotoxic Al^{3+} (Pineros and Kochian, 2009). This phenomenon is consistent with the explanation given earlier by Kochian (1995).

Field trial

The original soil chemical properties

Table 1 shows the chemical characteristics of the soils by depth at selected locations of the experimental plots in the trial before treatment. The topsoil pH was low; the values were even lower at the depth below 50 cm. At the depth of 45-60 cm, the pH values were lower than 3.5 in all the two locations in the experimental plot (Table 1). This low pH is consistent with the presence of jarositic mottles in the soils at that depth. Jarosite was observed in the topsoil during soil sampling.The low pH was also consistent with the presence of high exchangeable Al, especially at depth below 45 cm, which was the sulfuric layer. On the contrary, in the Kelantan Plains, the exchangeable Ca and Mg were very low (Soo, 1975). Hence, liming is necessary to supplement these macronutrients for rice production. We found that the peaty materials in the soils under experiment were completely decomposed as defined by soil taxonomy (Soil Survey Staff, 2010). The low CEC (data not shown) of less than 20 $cmol_c$ kg^{-1} soil further proved that the organic matter had decomposed and completely mixed with the mineral sediments. The CEC of normal organic matter is very high, having a value of more than 200 $cmol_c$ kg^{-1}. According to the Soil Taxonomy (Soil Survey Staff, 2010), these soils can be classified as Typic Sulfosaprists due to the presence of peaty materials and sulfuric horizon within the depth of 50 cm. The exchangeable Ca in the untreated topsoil ranged from 1.17 to 1.68 $cmol_c$ kg^{-1} soil, lower than the required level for rice of 2 $cmol_c$ kg^{-1} soil (Palhares, 2000). The exchangeable Mg was only 0.50-0.53 and its requirement is 1 $cmol_c$ kg^{-1} soil and Al concentration of 37-74 µM in the soil solution would cause toxicity to the growing rice plants in the field (Dobermann and Fairhurst, 2000). Based on the presence of the low amount of Ca and Mg, it is necessary that GML be applied at the appropriate rate to supply both elements which are required by rice.

Effects of lime treatment on soils

Soil analyses carried out on the soil samples after the first rice harvest showed erratic results (data not shown). This was due to the occurrence of flood during the Monsoon months. For instance, in T1, the topsoil pH, exchangeable Al, exchangeable Ca and exchangeable Mg were 3.95, 5.83, 1.06 and 0.46 $cmol_c$ kg^{-1} soil, respectively. In the T5, where 8 t ha^{-1} of GML were applied, the corresponding values were 4.38, 2.64, 2.86 and 1.21 $cmol_c$ kg^{-1} soil. Ironically, the respective values for T7 were higher than those of the T5, where only 4 t ha^{-1} of GML were applied. The corresponding values for this treatment were 4.93, 0.12, 8.60, 3.37 $cmol_c$ kg^{-1} soil. All these would be seen in the response of the rice plants shown by the yield of rice in this trial in the 1st season**.** However, the effect of this flood on rice yield was less remarkable in the 2nd season. The results of the soil analyses for the second season were as expected (Table 2). The lowest pH with a value of 3.95 was reported for the control. The highest pH, being 4.52, was reported for T5, where the most amount of GML was applied. Consistent with the lowest pH, the control treatment had the highest value of exchangeable Al, with a value of 12.75 $cmol_c$ kg^{-1} soil. As a result of the GML application, soil pH slowly increased, culminating in the T5 (Table 2).

Table 1. Some chemical properties of the soils at the trial before treatment.

Depth (cm)	T1 pH air-dried 1:2.5	T1 pH fresh 1:2.5	Al	Ca	Mg	K	O.C %	T3 pH air-dried 1:2.5	Al	Ca	Mg	K %	O.C
			--------------(cmol$_c$ kg^{-1})--------------						------------(cmol$_c$ kg^{-1})------------				
0-15	4.1	4.7	4.46	0.36	0.18	1.21	10.4	4.2	3.35	0.27	0.13	0.88	21.5
15-30	4.0	4.5	4.84	0.22	0.14	1.30	-	4.0	4.81	0.36	0.18	0.86	-
30-45	3.6	4.4	8.29	0.20	0.52	1.81	-	3.7	8.74	0.32	0.47	1.23	-
45-60	2.9	3.9	12.54	0.08	0.33	0.61	-	3.3	8.76	0.31	0.54	1.09	-
60-75	2.5	4.1	15.10	0.05	0.15	0.13	-	2.5	32.43	0.07	0.37	0.76	-

Table 2. Topsoil pH and exchangeable cations after ***second rice*** harvest.

Treatment	pH water 1:2.5	Al	Ca	Mg	K
		----------------------(cmol$_c$ kg^{-1})----------------------			
T1	3.95^{e}	12.75^{a}	1.58^{e}	0.48^{f}	0.41^{a}
T2	3.99^{e}	10.22^{ab}	1.99^{de}	0.57^{e}	0.24^{bc}
T3	4.06^{de}	9.45^{ab}	2.22^{cd}	0.70^{d}	0.15^{d}
T4	4.35^{b}	3.13^{c}	2.81^{b}	0.93^{b}	0.19^{bcd}
T5	4.52^{a}	2.37^{c}	3.74^{a}	1.10^{a}	0.17^{cd}
T6	4.21^{bc}	8.79^{ab}	2.57^{bc}	0.79^{c}	0.27^{b}
T7	4.16^{cd}	7.46^{bc}	2.47^{bc}	0.78^{cd}	0.21^{bcd}
LSD$_{0.05}$	0.14	5.15	0.47	0.08	0.08

Means followed by the same letter within a column are not significantly different (HSD P<0.05)

In this treatment, the exchangeable Ca and Mg were the highest in the trials, having values of 3.74 and 1.10 cmol$_c$ kg^{-1} soil, respectively. The increase in pH was concomitantly followed by the lowering of exchangeable Al in the soil, the value of Al being 2.37 cmol$_c$ kg^{-1} soil. This was the lowest value of exchangeable Al recorded for this trial.

Rice yield in the 1st season

The floods occurring during the experimental period had affected the rice seedlings in the field (Table 3). After the floods, some plots needed to be re-transplanted. There could also be removal of the some liming materials by the running water during the height of the flood period where each flood lasted for about a week. The effect of the flood is clearly seen in the erratic values of the rice yield (Table 3). There seemed to be no real difference in rice yield between treatments. The highest yield was seen on T2, where 2 t GML ha^{-1} was applied. But this yield was not significantly different from that of the control treatment.

Rice yield in the 2nd season

The highest rice yield for the 2nd season was 7.5 t ha^{-1} obtained by T6 (Table 3). For this treatment, 4 t GML^{-1} ha were applied in combination with 0.25 t ha^{-1} organic fertilizer (JITU™). Organic material is needed to accelerate the reduction of Fe that would result in faster pH increase (Muhrizal et al., 2006). Organic matter can also make Al inactive by chelating it (Muhrizal et al., 2003). The above yield is comparable to the yield of rice grown on good soils in the granary areas of the west coast states of Peninsular Malaysia. It was observed that the yield obtained by T6 was not significantly different from that of the T3, T4 and T5 (Table 3). There was an indication that applying 2 t GML ha^{-1} (T2) was not enough to ameliorate the soil for rice cultivation (Shamshuddin and Anda, 2012). As shown by the data in Table 2, for the T2, the pH was still low (3.99) and Al was very high (10.22 cmol$_c$ kg^{-1} soil). For T7, where 4 t GML ha^{-1} were applied in combination with fused magnesium

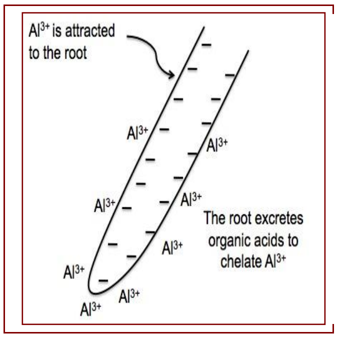

Fig 1. Postulated mechanism for rice plant in defending itself against A^{3+} toxicity (negative sign in the root indicates the negatively-charged surface).

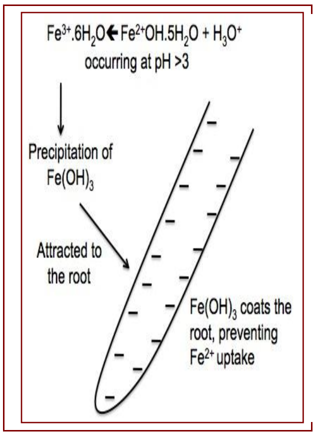

Fig 2. Postulated mechanism for rice plant in defending itself against Fe^{2+} toxicity (negative sign in the root indicates the negatively-charged surface).

phosphate, the yield was not significantly different from that of the T6. It means that instead of using organic fertilizer (compost), farmers in that area can apply lime together with fused magnesium phosphate. In this trial, rice roots affected by Fe toxicity were sampled and studied. These roots appeared to be reddish brown in color, indicating the presence of ferric hydroxides. The presence of ferric hydroxides on the surface of the roots of rice grown on acid sulfate soils of the Philippines had been identified by Moormann and van Breemen (1978).

Discussion

Soil pH and rice yield

It was found that the most suitable pH of water in the paddy field for growing MR 219 rice variety was about 6 (Elisa et al., 2011). This high pH level is uncommon in areas under acid sulfate soil condition (Shamshuddin et al., 2004a). Usually, the water pH is less than 4. For the area under investigation, the water pH was 3.7 (Merbok, Kedah). At low pH (< 5), Al dissolves, causing toxicity to the growing rice plants in the field (Elisa et al., 2011). For the current study, the Al concentration was more 800 µM (Merbok, Kedah) and this level is not uncommon for the acid sulfate soils in the country. The critical Al concentration for MR 219 rice variety was 15 µM (Elisa et al., 2011). To overcome the problem, we recommended application of ground magnesium limestone in combination with organic fertilizer (compost) at the rate of 4 t GML ha^{-1} plus 0.25 t organic fertilizer ha^{-1}. Beside pH increase, lime application can supply Ca and Mg to the growing rice. The Ca present in soils amended with either ground magnesium limestone is beneficial as Ca, to a certain extent, is able to reduce the toxic effect of Al^{3+} (Shamshuddin et al., 1991; Alva et al., 1986). For this trial, this had happened from T3 to T7 (Table 2). The alleviation of Al toxicity, should there be any, would be shown by the increase in the rice yield (Table 3). The presence of extra Mg could also contribute to the alleviation of Al toxicity as had been shown by Shamshuddin et al. (1991) for maize. Al^{3+} is toxic to plant and high exchangeable Al in soil is usually associated with low pH. This is shown by the data given in Table 2; the highest pH coincided with the lowest Al (T5). It showed the opposite effect in T1. As shown in the data in Table 1, the original exchangeable Al was extremely high in some samples, reaching a value of 32.43 $cmol_c\ kg^{-1}$ soil in the subsoil of T3. In the water in the vicinity of the experimental plots, Al would certainly exceed the critical level for rice production of 15 µM. (Elisa et al., 2011). This high Al in the solution can be reduced to an accepted level by applying GML at an appropriate rate. This study suggested that GML application at 4 t ha^{-1} would be appropriate. Fe toxicity is one of the most important problems facing production of rice on acid sulfate soils. In an abandoned rice fields near the study site (Kelantan), the water was reddish in color, indicating the presence of high amount of soluble iron. In this study, acid-extractable Fe in the soils was slightly above the critical level, ranging from 0.07 to 0.81 $cmol_c\ kg^{-1}$ soil (data not shown). Critical Fe concentration varies from 0.05 to 5.37 $cmol_c\ kg^{-1}$ soil (Dobermann and Fairhurst, 2000). Adding organic fertilizer (compost) a flooded acid sulfate soil would intensify the reducing condition, resulting in release of Fe^{2+}, which is toxic to rice plants (Tran and Vo, 2004). This reaction would also result in pH increase that helps precipitate Fe as hydroxides (Shamshuddin and Anda, 2012). Putting organic fertilizer alone at the rate applied in the current study did not cause significant effect on rice yield (Table 3). Treatment T6 in which GML applied together with organic fertilizer gave the highest rice yield of 7.5 t ha^{-1} in 2nd season (Table 3). On the contrary, high quality organic matter like organic fertilizer used in the current study would hasten reduction of Fe that result in quicker pH increase (Muhrizal et al., 2006). The lime (GML) used in this study was dolomitic limestone [$(Ca, Mg)(CO_3)_2$]. Adding this lime would increase soil pH accordingly, with concomitant addition of Ca and Mg into the soil (Auxtero and Shamshuddin, 1991). GML ameliorated in the soil according to the following reactions**:**

$$(Ca, Mg)(CO_3)_2 \rightarrow Ca^{2+} + Mg^{2+} + CO_3^{2-} \quad \text{(equation 1)}$$
$$CO_3^{2-} + H_2O \rightarrow HCO_3^- + OH^- \quad \text{(equation 2)}$$
$$Al^{3+} + 3OH^- \rightarrow Al(OH)_3 \quad \text{(equation 3)}$$

The GML dissolved readily on applying it into the acidic soil, releasing Ca and Mg (equation 1), and these macronutrients could be taken up by the growing rice plants. Subsequently, hydrolysis of CO_3^- (equation 2) would produce hydroxyls that neutralized Al by forming inert Al-hydroxides (equation 3).

Mechanism of Al tolerance

At the high rate of lime application, soil pH should be increased significantly, but not the case in this study (Table 2). When the soil was flooded to grow rice, water pH was also expected to rise (Muhrizal et al., 2006; Konsten et al., 1994). Unfortunately, water pH was still below 5. The equation below shows that Al^{3+} hydrolyses to produce acidity (pK_a of Al is 5):

$$Al^{3+}.6H_2O + H_2O \rightarrow Al^{2+}OH.5H_2O + H_3O^+$$

The rice plants there were not only subjected to Al^{3+} toxicity, but also H^+ stress. The combined effects can cause stunted growth of the rice and eventually the yield is very much reduced. In the field trial, the highest rice yield was in T6 where 4 t GML ha^{-1} was applied (Table 3). In this treatment, the exchangeable Al was 8.79 $cmol_c\ kg^{-1}$ soil. Some of the Al was precipitated as inert Al hydroxides due to pH increase by lime application, but a lot more remained in the soil and water. With this high exchangeable Al and low pH, we believed that the Al concentration in the water of the experimental plots could exceed the critical concentration of 15 µM. It means that the rice plants were growing under Al^{3+} stress. Yet we found that rice grew quite well, producing reasonable yield. It seemed that rice plants were able to defend themselves against Al^{3+} stress. There must a mechanism how the rice plants were able to reduce the effects of Al^{3+} toxicity. We believed that the rice plants have a special defense mechanism to somewhat reduce Al toxicity. A plausible mechanism for the reduction in Al toxicity is given in Figure 1. Al^{3+} was attracted to the negatively-charged cell walls of rice roots. When the Al^{3+} reached the cell walls the roots began to release citric, oxalic and malic acids. The higher the Al^{3+} on the cell walls, the more organic acids the rice roots would release. These acids, in turn, chelated the Al^{3+}, rendering it inactive. In this way, the rice plants were able to reduce the effects of Al^{3+} toxicity.

Table 3. Rice yields at the first and second harvest.

Treatment	First harvest ($t\ ha^{-1}$)	Second harvest ($t\ ha^{-1}$)
T1	4.5^{ab}	5.1^{bc}
T2	5.0^{a}	4.5^{c}
T3	3.5^{bc}	6.3^{abc}
T4	4.4^{abc}	6.6^{ab}
T5	4.2^{abc}	7.2^{a}
T6	3.7^{bc}	7.5^{a}
T7	3.1^{c}	6.8^{ab}
$LSD_{0.05}$	1.4	2.0

Means followed by the same letter within a column are not significantly different (HSD P<0.05)

Table 4. The rate of soil amendments used in the field trial

Symbol	Treatment
T1	Control (0 t GML^{+} ha^{-1})
T2	2 t GML ha^{-1}
T3	4 t GML ha^{-1}
T4	6 t GML ha^{-1}
T5	8 t GML ha^{-1}
T6	4 t GML ha^{-1} + JITU*
T7	4 t GML ha^{-1} + $FMP^{\#}$

+ GML – Ground magnesium limestone
* JITU – Organic fertilizer (0.25 t ha^{-1}) (compost)
\# FMP – Fused magnesium phosphate

How Fe^{2+} toxicity is reduced

When the experimental plots of the current study were submerged under water, Fe^{3+} in the soil was reduced to Fe^{2+} ion. The latter would be taken up by rice plants via their roots and Fe^{2+} is known to be toxic to rice plants (Tran and Vo, 2004). We can somewhat reduce the concentration of Fe^{2+} by accelerating reduction process that would result in earlier pH increase which, in turn, precipitate the Fe. While waiting for the pH to increase, the rice plants are subjected to Fe^{2+} stress. However, there is a process in the water that tend to reduce the uptake Fe^{2+} by rice roots, which can be considered as a mechanism for minimizing the effects of Fe^{2+} toxicity.

Like Al^{3+}, Fe^{3+} hydrolyses readily in water; the process is according to the following equation:

$$Fe^{3+}.6H_2O + H_2O \rightarrow Fe^{2+}OH.5H_2O + H_3O^+$$

The pK_a of Fe is 3. Note that the pH of water in the rice trial was higher than 3 due to GML treatment. Therefore, the reaction of Fe hydrolysis was in the reverse order. That means instead of producing proton, the reaction had resulted in the precipitation of Fe hydroxides. The slightly positively-charged $Fe(OH)_3$ is attracted to the negatively-charged cell walls of the rice roots. These hydroxides of Fe would appear as brown crust on the roots of rice (Figure 2). This explanation is consistent with that of Moormann and van Breemen (1978). The coating of rice roots would prevent further uptake of Fe^{2+}. Hence, the rice plants are spared from toxicity resulting from the presence of Fe^{2+}.

Materials and methods

Soils and their locations of sampling

For the laboratory experiment, soil and water were taken from a paddy field in Merbok, the northern state of Kedah, Peninsular Malaysia. This soil belonged to the Merbok Series, taxonomically classified as Typic Sulfaquept (Soil Survey Staff, 2010). A field trial was conducted at the Kemasin-Semerak Integrated Agricultural Development Project (IADP), Kelantan; this is in the northeastern part of Peninsular Malaysia where the soil was a Typic Sulfosaprist (Soil Survey Staff, 2010). At the onset of the growing season, the color of the water in the paddy fields in the Kelantan Plains is reddish, indicative of the presence of Fe. Both soils can be considered as acid sulfate soils as the pH is < 3.5 and exchangeable Al is very high, especially in the subsoil. Furthermore, pyrite and jarosite are present in the soils. The two sites are cropped to rice, but the yield is very low.

Plant material

Rice (*Oryza sativa*) variety MR 219 (indica) was used for both the laboratory experiment and field trial. This is the variety that is planted on about 90% of the granary areas in Peninsular Malaysia. From record, MR 219 rice variety planted on acid sulfate soils performed very poorly with yield about 2 t ha^{-1} per season, using farmer's practice, which is far below the national average of 3.8 t ha^{-1}.

Experimental

Laboratory experiment

This was a short-term experiment conducted in two phases:
Rice seeds were soaked in a hormone-based chemical (Zappa™), rinsed with distilled water and then left in the dark place for 24 hours. Three pre-soaked seeds were then transferred into test tubes containing 0.5 mM $CaCl_2$ solution with various concentrations of Al (0, 10, 20, 30, 40 and 50 µM) using $AlCl_3$. In the second phase a seed germination experiment was conducted using acid water containing high concentration of Al taken from a paddy field in Merbok, Kedah. The pre-soaked seeds were exposed to 100 mL of the acid water. For this experiment, the pH of the water was adjusted to various levels (3, 4, 5, 6 and 7) using 0.01 M HCl or 0.01 M NaOH. The seeds were allowed to germinate in tubes and let to grow into seedlings for 7 days. No fertilizers were added to accelerate the growth. The rice seedlings were harvested and the solution in each tube was filtered using 0.45 µm milipores filter. These solutions were analyzed for

the presence of organic acids by HPLC, using Aminex HPX-87H column. Filtrate samples (100 µL) were injected into the HPLC using a glass syringe and eluted isocratically with 0.008 N H_2SO_4 at a constant flow rate of 0.6 mL/minute for 25 minutes at 20°C. Peaks for the organic acids were detected at a wavelength of 210 nm and the organic acids 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 was calculated and expressed as µM.

Field trial

This trial was laid out in the field using a completely randomized design, with five replications. There were altogether seven treatments (Table 4). The treatment for the trial included a control (no lime, T1); the rest of the treatments are given in Table 4. The amount of organic fertilizer (compost) applied was 0.25 t ha^{-1}, the standard rate applied on paddy field. For this trial, two successive crops of rice were planted. Ground magnesium limestone was applied once and the seeding was done two weeks later, just before irrigation water was allowed into the experimental plots. Standard fertilizer rates were given to the growing rice plants in the field (90-120 kg N ha^{-1}, 12-18 kg P ha^{-1}, 90-120 kg K ha^{-1}), using urea, NPK Blue (12:12:17+TE) and NPK Green (15:15:15+TE) as the sources of the nutrients. At harvest, rice yield (in terms of t ha^{-1}) for each experimental was determined using standard method. The roots of the rice affected by Fe toxicity (stunted roots) were sampled to study the reddish materials coating them.

Soil analysis

Soil pH (in water) was determined by a pH meter. Basic exchangeable cations were extracted by NH_4OAc, buffered at pH 7. Exchangeable Ca, Mg, and K in the NH_4OAc extract were determined by atomic absorption spectrophotometry (AAS). Exchangeable Al was extracted by 1 M KCl and determined by AAS. The organic carbon was determined by the standard Walkley-Black method. Available P was determined by the method of Bray and Kurtz (1945). Iron in the soils was determined by double acid method (henceforth referred to as acid-extractable Fe). It was extracted using 0.05 M HCl in 0.0125 M H_2SO_4. A five-gram 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 AAS.

Statistical analysis

Statistical analysis for means comparison was carried out by the Tukey's test (HSD) and/or LSD 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.

Conclusions

The data obtained in the present laboratory and field experiments showed that due to GML application, rice variety MR 219 grew productively even if the water pH was below 5 and Al concentration was more than 15 µM. This is probably due to rice having a special defense mechanism under Al^{3+} stress. When Al^{3+} ions were on the cell walls of the rice roots, organic acids were excreted which chelated the Al^{3+}, rendering them inactive. In this way, the rice plants were able to defend themselves against Al^{3+} toxicity. As for the reduction of Fe^{2+} toxicity, the results obtained in the present study show another mechanisms were involved. When GML was applied onto the soil, water pH increased to above 3. When this happened the reverse of the hydrolysis of Fe^{3+} had occurred that would result in the precipitation of $Fe(OH)_3$. This slightly positively-charged Fe hydroxide had been attracted to the negatively-charged cell walls of the rice roots as shown by brown crusts of Fe hydroxides coating the rice root surfaces, preventing further uptake of Fe^{2+}.

Acknowledgements

The authors would like to thank Universiti Putra Malaysia and the Ministry of Higher Education Malaysia (under LRGS Program – Food Security) for financial and technical support. We acknowledge the help of Emeritus Profesor K.M. Goh of Lincoln University, NZ for reading and improving the manuscript.

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