Ilmu Pertanian (Agricultural Science)
http://journal.ugm.ac.id/jip
Vol. 7 No. 1 April, 2022: 47–55| DOI: doi.org/10.22146/ipas.64252

The potential of organic matter and water management on the alleviation of iron toxicity in rice plants

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Article Info

Received : 22nd February 2021
Revised : 7th May 2021
Accepted: 11th May 2021

Keywords:
Actual acid sulfate soil, iron toxicity, leaching, organic matter, potential acid sulfate soil

Abstract

Iron toxicity in rice plants occurs due to the excessive concentrations of ferrous ions (Fe^{2+}) in the soil solution. The application of organic matter and water management is one way to alleviate iron toxicity in rice plants. This study aimed to determine the potential of organic matter and water management in controlling the solubility of Fe^{2+} in the soil and its effect on the symptoms of toxicity, growth and yield of rice plants. The research was conducted in the greenhouse of the Indonesian Swampland Agricultural Research Institute from August to December 2020, arranged in a completely randomized design with three factors. The first factor consisted of low Fe^{2+} concentration (<300 ppm, actual acid sulfate soil (AASS)) and high Fe^{2+} concentration (>300 ppm, potential acid sulfate soil (PASS)), the second factor consisted of waterlogging without leaching and leaching of once every two weeks, and the third factor consisted of without organic matter amendment and with organic matter amendment of 2.5 ton.ha^{-1}. The results showed that the soil leaching in acid sulfate soils decreased the activity of PPO by 10.28 % and increased yield by 10.10 %. Meanwhile, the application of organic matter in acid sulfate soil decreased the activity of PPO by 8.91 % and increased yield by 8.06 %. The leaching of once every two weeks and organic matter amendment of 2.5 ton.ha^{-1} are recommended to alleviate iron toxicity and increase rice productivity in acid sulfate soil.

INTRODUCTION

Iron toxicity in plants is caused by a high concentration of Fe^{2+} in the soil solution. The concentration of Fe^{2+} increases due to the reduction process of iron from ferric (Fe^{3+}) into ferrous (Fe^{2+}) ions. In waterlogged conditions, the amount of oxygen becomes strictly limited, and the rate of oxygen diffusion is 10,000 times slower than under an aerobic condition so that other inorganic compounds is used as electron acceptor by microorganisms to sustain their life (Susilawati and Nursyamsi, 2013). Under anoxic condition, Fe^{3+} ions transform to Fe^{2+}. The reduction of Fe^{3+} ions is largely influenced by the process of microbial activity and organic matter as an electron donor (Annisa and Nursyamsi, 2016a).

Iron is an essential element for the growth of rice. Rice plants grow well at Fe concentration range of 100 ppm to 300 ppm, at low Fe concentration of 10 ppm to 30 ppm, or at the Fe toxicity of >300 ppm

How to cite: Nugroho, N., Kurniasih, B., Utami, S.N.H., and Yusuf, W.A. (2022). The potential of organic matter and water management on the alleviation of iron toxicity in rice plants. *Ilmu Pertanian (Agricultural Science)*, 7(1), pp. 47–55.

ISSN 0126-4214 (print) ISSN 2527-7162 (online)

(Mahender et al., 2019). Iron toxicity in rice plants reduces biomass, inhibits root growth, and causes leaf bronzing and necrosis (Zhang et al., 2018). Under the excess of Fe condition, rice plants develop a resistance mechanism of exclusion and inclusion, which is through the inhibition of the Fe uptake in roots by Fe^{2+} oxidation to Fe^{3+} in the rhizosphere, holding the Fe in the root tissues and reducing translocation of Fe from roots to shoots, compartmentation or storage in the leaves and detoxify of enzyme in the symplast (Mahender et al., 2019)

Organic matter and water management can potentially control the solubility of Fe in the process of oxidation-reduction. Organic matters function to maintain the condition of reductive soil and chelation of the toxic elements in acid sulfate soil. The provision of organic matter such as compost with C/N < 20 can decrease the concentration of Fe^{2+}. This occurs because the chelation process is more intensive than the reduction process of Fe^{3+} to Fe^{2+} by organic acids. On other hand, organic matter with C/N > 20 tends to increase the concentration of Fe^{2+} because it promotes the reduction of Fe^{3+} to Fe^{2+} (Susilawati and Nursyamsi, 2013).

Acid sulfate soils under waterlogged conditions promotes the reduction of Fe^{3+} to Fe^{2+}, so the concentration of Fe^{2+} increases to reach thousands of $mg.L^{-1}$ in the soil solution. This phenomenon occurs mainly in an actual acid sulfate soil, which is waterlogged by rainwater or high tide. In order to reduce the concentration of toxic Fe^{2+}, leaching is necessary by removing water from the rice fields, then flooding it again with high tide. In addition to reducing the concentration of Fe^{2+}, leaching also reduces toxic compounds such as SO^{4-} and H^{+} (Ar-riza et al., 2015). This research aimed to find the potential of organic matter application and water management in controlling the solubility of Fe^{2+} in the soil and its effect on the symptoms of toxicity, growth and yield of rice plants.

MATERIALS AND METHODS

The research was conducted in the greenhouse of the Indonesian Swampland Agricultural Research Institute (ISARI) from August to December 2020, arranged in a randomized block design of three factors with three replications. The first factor was the concentration of soil Fe, consisting of low concentration of Fe^{2+} (<300 ppm) (F1) and high concentration of Fe^{2+} (>300 ppm) (F2). The second factor was water management, consisting of waterlogging without leaching (P1) and leaching every 2 weeks (P2). The third factor was the type of organic matter, consisting of without organic matter (B1) and with organic matter of 2.5 $ton.ha^{-1}$ straw+rush weed compost (B2). The rice variety used was Inpara 5.

Acid sulfate soil used was taken from the abandoned land/fallow in Roham Jaya Village (F1) to represent actual acid sulfate soil (AASS) and from an intensive rice cultivation land in Tanjung Harapan Village (F2) to represent potential acid sulfate soil (PASS). The soil samples were taken at a tillage depth of 0 cm to 20 cm. The treatment of leaching was set up by removing water in the pot until no droplets of water left in the pot. Then waterlogging was done again on the pot as high as 5 cm using rainwater. The leaching was done once every two weeks. For the treatment without leaching, a 5 cm puddle was kept constantly during the growth of the plants. Organic matters in situ in the form of straw and rush weed (*Eleocharis dulcis*) were taken from the acid sulfate soil of South Kalimantan. Organic matters with the composition of 50 % straw and 50 % rush weed were composted aerobically using *Tricoderma* decomposer before application.

A total of 120 fruit pots were prepared as a growing medium. Each pot was filled with 8 kg of soil. The soils were fertilized using a 300 $kg.ha^{-1}$ of urea by 2/3 part seven days after planting (DAP) and 1/3 part 30 DAP, whilst a 200 $kg.ha^{-1}$ of SP36 and a 100 $kg.ha^{-1}$ of KCl was given at 7 DAP.

The variables observed include the concentration of Fe, pH, Eh of soil, root volume, dry weight of plant, leaf bronzing, polyphenol oxidase (PPO) enzyme, yield component and grain yield per hill on the water content of 14 %. Observation of the PPO enzyme referred to Mehraban et al. (2008). Observation of soil and plant referred to Balai Penelitian Tanah (2009), the concentration of Fe was observed by extraction with NH_4OAc_1M pH 4.8 then measured using an Atomic absorption spectrometry, pH and Eh were observed using reference electrode, while the dry weight was determined using a gravimetric method. The data obtained were analyzed using analysis of variance (ANOVA), and the differences between treatments were evaluated using Duncan Multiple Range Test (DMRT, α= 5 %). Analysis of variance and correlation analysis were performed using SAS 9.4 and SPSS 16 for windows, respectively.

RESULTS AND DISCUSSION

Chemical properties of soil and organic matter

This research used soil with low Fe concentration (F1) and high Fe concentration (F2). Based on the Soil Survey (2014), the F1 soil is actual acid sulfate soil (AASS) type, classified as Typic sulfaquept, with a pH of 2.86 (extremely acidic), C/N ratio of 10.23 and brownish color. Meanwhile, the F2 soil is a potential acid sulfate soil (PASS) type, classified as Typic Sulfaquent, with a pH of 3.96 (extremely acidic), C/N ratio of 17.75, and gray color (Table 1). The PASS can turn into an actual acid sulfate soil if it experiences oxidation due to drainage or excessive dryness. In contrast, the AASS can also turn into the potential acid sulfate through waterlogging, drying, leaching, and organic matters amendment (Nursyamsi et al., 2014).

The organic matters used were rush weed and straw compost (1:1) with a pH of 5.02, organic C of 45.11, total N of 2.11 %, total P of 0.39 % and total K of 0.19 %. The rice straw and rush weed (*Eleocharis dulcis*) are a major source of organic matters for acid sulfate soils area. Farmers in South Kalimantan and Central Kalimantan traditionally do the management of organic matters by means of *tajak-puntal-hambur* system. *Tajak* means slashing/ clearing land using *tajak*, which is a type of long sword. Weeds or rice straw resulted from the slash are collected in spots by forming balls, and this work is called puntal or spinning. The collection of organic material is left for about one month until composting occurs. Then the organic material is chopped or separated to be spread on the surface of the soil, and this work is called scattering or hambur (Noor and Rahman, 2015). Organic matters given can be in the form of compost or raw, and this method is more beneficial for plant growth because the return of rice straw, crop residues and rush weed means restoring the nutrients contained in the organic matter.

The dynamics of Fe^{2+}, pH, and Eh in the treatment of leaching and organic matter in acid sulfate soils

The dynamics of Fe^{2+} in AASS (F1) and PASS (F2) showed different patterns. In the AASS with the low concentration of Fe, the concentration of Fe^{2+} increased at 2 weeks after planting (WAP) (Figure 1). The increase occurred due to the high concentration of Fe^{3+} (Fe dithionite 2.37 %, Fe pyrophosphate 0.19 %, and Fe oxalate 4.33 %) that could be reduced (Table 1). At 2 WAP, the increase in the concentration of Fe^{2+} was lower in the soil with organic matter (B2) amendment. Because of the application of organic matters such as compost that was relatively mature (C/N <20), the chelation process was more intensive than the reduction process of Fe^{3+} to Fe^{2+} by organic acids (Susilawati and Nursyamsi, 2013).

In the soil with leaching (P2), the concentration of Fe^{2+} decreased at 2 WAP because the dissolved Fe^{2+} ions were carried by the water flow, but the fluctuations increased at 6 WAP (Figure 1). This could happen because during the leaching and rewater-logging, oxygen was trapped due to the hydrostatic pressure, and there was an infiltration of oxygenated water. The concentration of Fe^{2+} decreased as the influx

Table 1. Soil chemical properties

Parameter	Low concentration of Fe^{2+}	High concentration of Fe^{2+}
Fe^{2+} (ppm)	123.35	2298.72
Fe dithionite (%)	2.37	0.60
Fe phrophosphate (%)	0.19	0.23
Fe oxalate (%)	4.33	1.68
pH	2.86	3.96
Eh (mV)	346.53	-191.58
Organic-C (%)	5.83	2.84
Total-N (%)	0.57	0.16
C/N ratio	10.23	17.75
Available-P (ppm)	27.03	8.18
Exchangeable-K (cmol(+).kg^{-1})	0.31	0.18

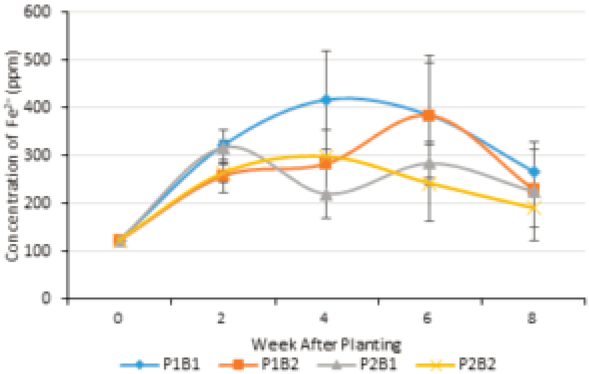

Figure 1. Concentration of Fe^{2+} by organic matter and water management in actual acid sulfate soil (F1) with low initial concentration of Fe^{2+}, P1B1= without leaching and without organic matter, P1B2= without leaching and with organic matter, P2B1= leaching and without organic matter, P2B2= leaching and with organic matter.

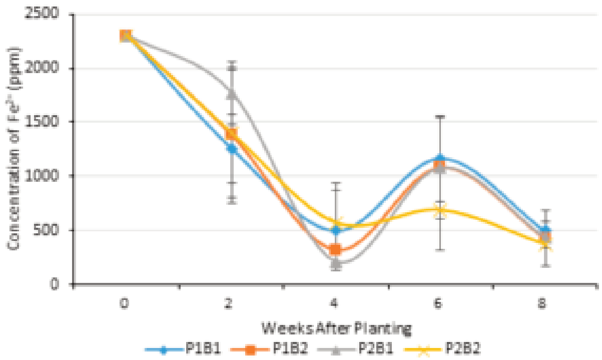

Figure 2. Concentration of Fe^{2+} by organic matter and water management in potential acid sulfate soil (F2) with high initial concentration of Fe^{2+}, P1B1= without leaching and without organic matter, P1B2= without leaching and with organic matter, P2B1= leaching and without organic matter, P2B2= leaching and with organic matter.

of oxygenated surface water entering the aquifer and oxidizing the Fe^{2+}. When the soil was rewaterlogged, oxygen source was disconnected, and the reduction and concentration of Fe^{2+} increased (Zhang et al., 2020). However, the increase in the concentration was less than that in the soil waterlogged continuously. As reported by Khairullah et al. (2011), the ability to reduce the Fe^{2+} level is the highest in the plots with intermittent water systems (waterlogged and dried alternately), and the lowest when waterlogged continuously.

The increase in the concentration of Fe^{2+} in the waterlogged soils might keep increasing, and at a climax point, a decrease in the concentration of Fe^{2+} would occur. A decrease in the concentration of Fe^{2+} after reaching the highest point was due to a reduced availability of Fe^{3+}, which could be reduced in the subsoil (Susilawati and Fahmi, 2013). After the 6 WAP, there was a decrease in the concentration of Fe^{2+} in the treatment of waterlogging (P1). The dynamic of Fe^{2+} concentration in the AASS (F1) showed the lowest increase found in the leaching dan organic matter treatment (P2B2) from 123.35 ppm to 190.51 ppm (Figure 1).

In the PASS with high concentration of Fe^{2+}, the pattern seen was a decrease in the concentration of Fe^{2+} (Figure 2). This was due to the less reduced Fe^{3+}, the presence of precipitation of carbon and sulfide (Reddy and Delaune, 2008), and the deposition by water forming Fe hydroxide into the plaque on the root surface (Shamshuddin et al., 2013). The combination of leaching and organic matter treatment caused a high decrease in the concentration of Fe^{2+} from 2298.72 ppm to 370.15 ppm. The organic acids produced from the decomposition of organic matter in the soil solution could bind Fe^{2+} in the soil to form chelate so that more concentration of Fe^{2+} could be leached.

The condition of the oxidative and reductive soil was indicated by the value of the redox potential (Eh). The increased content of soil organic matter reduced the soil redox potential value caused by the consumption of oxygen in a large amount during the oxidation processes that occurred in the soil to form organic compounds so that the soil conditions became increasingly reductive (Annisa and Nursyamsi, 2016b).

The same pattern was indicated in both AASS (F1) and PASS (F2), in which a reduction in redox potential occurred at 2 WAP. In AASS, the Eh kept decreasing, while in PASS, there was an increase at 6 WAP and decrease at 8 WAP (Figure 3). It is also associated with the development of the roots of rice plants. The rice plants were able to oxidize the area around its roots so that Fe^{2+} were oxidized to Fe^{3+}. Fe^{2+} oxidation occurs because plant roots respond to high Fe^{2+} concentrations in the soil by releasing O_2 in the rhizosphere (Mahender et al., 2019).

The reduction process requires H+ ions that are equivalent to the amount of Fe^{2+} or H_2S formed. Under reductive condition, the Eh decreased, while the pH increased because the H^+ ions were released so that the pH increased. In this research, the same pattern was shown in AASS (F1) and PASS (F2), in which pH increased at 2 WAP (Figure 4), which was in line with the decrease in the Eh. The value of Eh and pH were negatively correlated (Napisah et al., 2020).

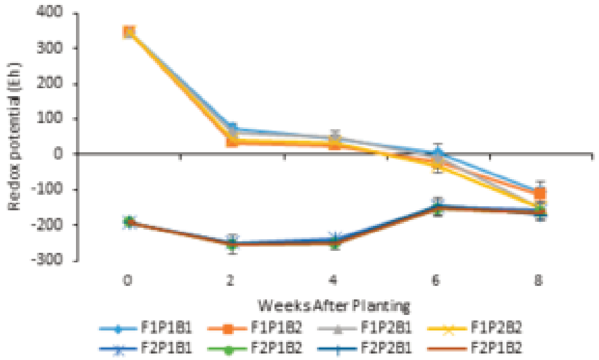

Figure 3. The Eh value of soil with organic matter and water management in acid sulfate soils, F= concentration of Fe^{2+} (F1: low concentration of Fe^{2+} (<300 ppm), F2= high concentration of Fe^{2+} (>300 ppm), P= water management (P1= waterlogging without leaching, P2= leaching every 2 weeks, B= organic matter (B1= without organic matter, B1= with organic matter).

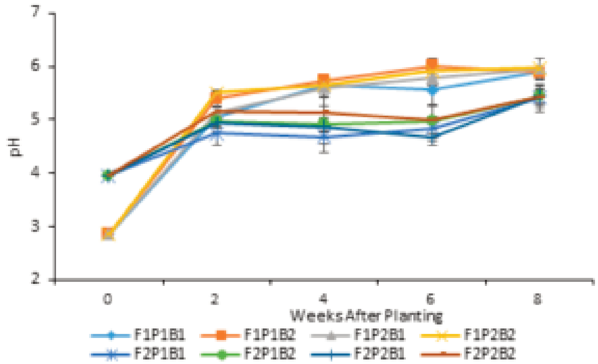

Figure 4. The pH value of soil with organic matter and water management in acid sulfate soils, F= concentration of Fe^{2+} (F1= low concentration of Fe^{2+} (<300 ppm), F2= high concentration of Fe^{2+} (>300 ppm, P= water management (P1= waterlogging without leaching, P2= leaching every 2 weeks, B= organic matter (B1= without organic matter, B1= with organic matter).

The potassium content and organic carbon in the soil

Soil leaching increased the potassium (K) content of the soil to 0.624 cmol(+).kg^{-1}, significantly higher than without leaching (Table 2). This result occurred because leaching can increase CEC of the soil, so that nutrients are available for plants. Leaching causes the decomposition of organic matter in the soil to produce organic acids more rapidly than continuous flooding. Humic acid forms carboxylic (-COOH) and phenolic (-OH) as the source of a negative charge increasing the CEC (Herviyanti et al., 2012).

There was no interaction between treatments on soil organic carbon (SOC) content. The addition of organic matter increased the SOC to 11.31 % (Table 2). SOC is used as an energy source to liberate P and S into a form available to plants through the enzyme hydrolysis process (Saidy, 2018). Increased SOC will decrease the concentration of Fe due to the formation of the complex reaction of Fe-organic. Thus, the binding of P with Fe cation is reduced, and P becomes more widely available (Herviyanti et al., 2012).

The volume and surface area of roots

Iron toxicity damages the plants by first inhibiting the growth of plant roots. The excess of Fe^{2+} ions could increase the production of H_2O_2 in the root tip and inhibit the growth of primary roots (Reyt et al., 2015). It would change the architecture of the root, such as the reduced number, length, angle, volume and diameter and root hairs (Li et al., 2016). There

Table 2. Potassium and soil organic carbon with organic matter and water management in acid sulfate soils

Treatments	K-dd (cmol(+).kg^{-1})	Organic-C (%)
Concentration of Fe^{2+}		
Low concentration of Fe^{2+} (<300 ppm)	0.585 a	12.64 a
High concentration of Fe^{2+}(>300 ppm)	0.488 a	8.49 b
Water management		
Without leaching	0.449 n	10.43 m
With leaching	0.624 m	10.70 m
Organic matter		
Without Organic matter	0.540 p	9.82 q
With Organic matter	0.533 p	11.31 p
Interaction	(-)	(-)

Remarks: Means followed by the same letters in the same column are not significantly different according to DMRT α= 5 %. The (-) indicates no interaction between factors.

Table 3. Root volume (cm^3) and surface area (cm^2) with organic matter and water management in acid sulfate soils

Treatments	Root volume (cm^3)	Root surface area (cm^2)
Concentration of Fe^{2+}		
Low concentration of Fe^{2+} (<300 ppm)	76.67 a	146.36 a
High concentration of Fe^{2+}(>300 ppm)	68.33 b	123.32 b
Water management		
Without leaching	70.83 m	131.32 m
With leaching	74.17 m	138.36 m
Organic matter		
Without Organic matter	69.58 q	123.99 q
With Organic matter	75.42 p	145.68 p
Interaction	(-)	(-)

Remarks: Means followed by the same letters in the same column are not significantly different according to DMRT α= 5 %. The (-) indicates no interaction between factors.

Table 4. Correlation between observed variables

Treatments	CFe	KCS	SOC	RV	RSA	LBI	PPO	GY
CFe	1	-0.707’’	-0.249	-0.731’’	-0.438’	0.525’’	0.680’’	-0.775’’
KCS		1	0.478’	0.616’’	0.611’’	-0.646’’	-0.743’’	0.793’’
SOC			1	0’423’	0.483’	-0.502’	-0.296	0.434’
RV				1	0.632’’	0.414’	-0.468’	0.627’’
RSA					1	-0.201	-0.469’	0.642’’
LBI						1	0.550’’	-0.585’’
PPO							1	-0.868’’
GY								1

Remarks: CFe= concentration of Fe^{2+}, KCS= potassium content in soil, SOC= soil organic carbon, RV= root volume, RSA= root surface area, LBI= leaf bronzing index, PPO= activity of polyphenol oxidase, GY= grain yield per hill, (’)= correlation is significant at the α= 5 %, (’’)= correlation is significant at α= 1 %.

was no interaction between the treatment of Fe^{2+} concentration, water management and organic matter on the volume and surface area of roots. The largest root volume and surface area was found in acid sulfate soils with low concentrations of Fe^{2+}. The application of organic matter increased the surface area and volume of roots (Table 3). Root volume and surface area was significantly correlated with soil organic carbon (r= 0.423 and r= 0.483) (Table 4).

The leaf bronzing index and activity of PPO enzyme

The typical visual symptoms associated with the toxicity of Fe is rice leaf bronzing (Mahender et al., 2019). Symptoms of bronzing begins at the older leaves that are fully developed with the appearance of small brown spots spread from the tips of the leaves to the base. The subsequent symptoms are the leaf tips turning yellow orange and being dried. The highest leaf bronzing index was found in the high Fe^{2+} concentration treatment (Table 5). Leaf bronzing is related to the activity of the polyphenol oxidase (PPO) enzyme. The role of the PPO in the symptoms of bronzing was the release of oxidized polyphenol, a compound responsible for bronzing due to the toxicity of Fe. In this research, the increase in activity of PPO enzyme was found in the high concentration of Fe^{2+} (Table 5). Saikia and Baruah (2012) also found an increasing trend of PPO under the influence of higher Fe^{2+} in the medium. The PPO catalyzes many oxidation reactions of compounds such as phenol into a quinone. The PPO was also

Table 5. Leaf bronzing index and activity of PPO enzyme with organic matter and water management in acid sulfate soils

Treatments	Leaf bronzing index	Activity of PPO enzyme (unit)
Concentration of Fe^{2+}		
Low concentration of Fe^{2+} (<300 ppm)	0.18 b	13.46 b
High concentration of Fe^{2+} (>300 ppm)	0.24 a	18.28 a
Water management		
Without leaching	0.22 m	16.73 m
With leaching	0.19 m	15.01 n
Organic matter		
Without Organic matter	0.22 p	16.61 p
With Organic matter	0.20 p	15.13 q
Interaction	(-)	(-)

Remarks: Means followed by the same letters in the same column are not significantly different according to DMRT α= 5 %. The (-) indicates no interaction between factors.

Table 6. Grain yield per hill (gram) and harvest index with organic matter and water management in acid sulfate soils

Treatments	Grain yield per hill (g)	Harvest index
Concentration of Fe^{2+}		
Low concentration of Fe^{2+} (<300 ppm)	21.63 a	0.47 a
High concentration of Fe^{2+}(>300 ppm)	15.82 b	0.43 b
Water management		
Without leaching	17.82 n	0.44 n
With leaching	19.62 m	0.46 m
Organic matter		
Without Organic matter	17.99 q	0.44 q
With Organic matter	19.44 p	0.46 p
Interaction	(-)	(-)

Remarks: Means followed by the same letters in the same column are not significantly different according to DMRT α= 5 %. The (-) indicates no interaction between factors.

involved in the synthesis of lignin, a compound of the cell wall containing phenolic cluster. The PPO was reported to play an important role in plant defense mechanism and oxidative stress.

Soil leaching decreased PPO activity by 10.28 % (Table 5). The activity of PPO was negatively correlated with soil potassium content (r= -0.743) (Table 4). Symptoms of iron poisoning were associated with excess Fe^{2+} ions in plant tissues (Dobermann and Fairhurst, 2000). Potassium in iron-stressed fields can reduce symptoms of iron toxicity (Gao et al., 2014). Unoki et al. (2020) also reported that application of 100 $kg.ha^{-1}$ K_2O increased the tolerance of the IR64 variety to iron toxicity. Potassium increased iron exclusion from roots and reduced iron translocation to the shoot. The treatment with organic matter also reduced PPO activity by 8.91 % (Table 5). PPO activity correlated with root surface area (r= -0.469) and volume (r= -0,468) (Table 4). Root growth will affect the ability of roots to store iron in roots. Root growth is inhibited due to increased reactive oxygen in roots will affect ferritin function. Ferritin is an iron storage protein. Ferritin contributes to protect plants from oxidative stress caused by excess Fe. Loss of ferritin function can reduce Fe levels in roots. Fe will be translocated to the shoot (Reyt et al., 2015).

The grain yield per hill and harvest index

There was no interaction between treatment of Fe^{2+} concentrations, water management and application of organic matter on grain yield per hill and harvest index. The higher yields per hill and harvest index was found in acid sulfate soils with low Fe^{2+} concentrations (Table 6). According to the research of Suswanto et al. (2007), when the solubility of Fe^{2+} in the soil increases, the yield of rice decreases.

Soil leaching increased grain yield per hill by 10.10 % compared to without leaching (Table 6). Grain yield per hill in the leaching treatment increased because the soil potassium content increased (r = 0.793) thus increasing root oxidation and decreasing PPO enzyme activity (r= -0.868). According to the research of Ar-riza et al. (2015), leaching treatment in acid sulfate wetlands increased dry grain yield by 1.9 ton.ha^{-1} to 2.8 ton.ha^{-1} compared with no leaching. Meanwhile, the provision of organic matter increased the yield of grain per hill by 8.06 % compared to without organic matter (Table 6). Khairullah et al. (2011) stated that the provision of organic matter, straw, and rush weed compost could improve the rice yield in acid sulfate soil. The grain yield per hill on the treatment of organic matter increased in relation to the increased surface area (r= 0.642) and root volume (r= 0.627), and decreased PPO activity (r= -0.868) (Table 4).

CONCLUSIONS

There was no interaction between treatment of water management and application of organic matter on iron toxicity and yield of rice plant in acid sulfate soil. The soil leaching in acid sulfate soils decreased the activity of polyphenol oxidase by 10.28 % and increased yield by 10.10 %. Meanwhile, the application of organic matter in acid sulfate soil decreased the activity of polyphenol oxidase by 8.91 % and increased yield by 8.06 %. The application of organic matters at 2.5 ton.ha^{-1} and water management with leaching treatment once every two weeks are recommended to reduce the symptoms of iron toxicity and increase the yield of rice plants in acid sulfate soil.

ACKNOWLEDGMENTS

The authors would like to express deepest gratitude to the Head of Indonesian Swampland Agricultural Research Institute and its staff for providing assistance and facilities for this research.

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