Swampy rice lines for iron toxicity tolerance and yield components performance under inland swamp at Sorong, West Papua, Indonesia

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Abstract. *Maruapey A, Wicaksana N, Karuniawan A, Windarsih G, Utami DW. 2020. Swampy rice lines performance for iron toxicity tolerance and yield components under inland swamp at Sorong, West Papua, Indonesia. Biodiversitas 21: 5394-5402.* The extensification of sub-optimal lowland farming strategy is expected to support the increase of rice production. Based on the efforts, the development of tolerant rice varieties to Fe-toxicity to be used in the swampy land area is one of the approaches for increasing rice production. Currently, promising rice lines that are tolerant to Fe-toxicity has been developed using molecular breeding approach for inland swampy area. The objective of this research was to evaluate the performance of 15 promising rice lines that have various genetic backgrounds in inland swamp in Sorong, West Papua. The field experiment was conducted during the second rice planting season (August 2018 to February 2019) to evaluate the performance of the promising rice lines to the morpho-agronomic performance under the lowland swamp conditions. The trial was laid out in a Randomized Complete Block Design (RCBD) consisting of 4 m x 5 m square plots, with 25 cm x 25 cm planting distance and 3 replications. The results revealed that most of the lines had good performances on the Fe-toxicity tolerance, based on bronzing, root length, and biomass characters. The G1 line had the best performance on yield component characters, especially the panicle length. This line also had the highest grain yield (6.15 ton.ha^{-1}) followed by the next promising line of G7 (5.92 ton.ha^{-1}). The genetic performance of these lines showed that they contained *IRT* (*Iron Regulation Transporter*) alleles that contributed to partitioning the Fe tolerance mechanism.

Keywords: Fe-toxicity tolerant, inland swamp, promising lines of molecular breeding, yield characters

INTRODUCTION

The national food needs to keep increasing along with the increase of population. Meanwhile, one of the serious threats for national food security is the conversion of intensive rice fields into non-agriculture, especially for road infrastructure, airports, office buildings, and industry (Irawan 2005; Mulyani 2017). In facing climate change and extreme weather conditions, Indonesia needs to find ways to increase crop production (Naylor et al. 2007). Therefore, the strategy of utilizing the sub-optimal lands, such as swamps, becomes one of the solutions to increase national food production.

The swampland areas in Indonesia are classified into two main categories, i.e. tidal swamp and inland swamp. These two types of swamp are dominantly distributed in Sumatra, Kalimantan, Papua, and a small part in Sulawesi. Total swamp area in Indonesia is estimated to be 34 million ha, accounting for 18% of Indonesia's land area, of which 20.09 million ha (60.2%) are tidal swamp and 13.30 million ha (39.8%) are of inland swamp (Departement of Public Works 2009; Sulaiman et al. 2019). The inland swamp is a land area that has a puddle at least for three months throughout the year with a height of at least 50 cm.

The use of inland swamp for agricultural development is termed as the rainwater swampland (Noor 2007). So, this type of land was characteristically flooded for a long period of time and the soil is basically muddy. Based on the water level and the length of puddle, an inland swamp in Indonesia consists of (a) a shallow rainwater swamp with a puddle height of <50 cm for <3 months, (b) a middle inland swamp with a puddle height of 50-100 cm for <6 months, and (c) a deep inland swamp with a puddle height of >100 cm during >6 months (Widjaja et al. 1992).

The frequency of puddle occurs relatively long, so that the exposure of plants to iron occurs continuously. The iron (Fe^{2+}) toxicity is one of the important abiotic stresses which could decrease rice production. The results of evaluation of the tolerance level of some swamp rice varieties to Fe^{2+} toxicity showed that, in general, the rice plants experienced the symptom of sensitive moderate bronzing (score 3-4), such as in varieties of Inpara 3, IR42, Banyuasin, Batanghari, and Mahsuri. Among these varieties, Mahsuri variety showed the lowest bronzing score (Utami 2018).

The understanding of the Fe-toxicity mechanism showed that there are some different types of Fe-toxicity tolerance mechanisms in rice plants. Type I consists of the Fe exclusion from roots, and uses the aerenchyma-derived

oxygen or the enzymatic activity to oxidize Fe^{2+} into Fe^{3+}, which precipitates as Fe plaque at the root surface (Wu et al. 2014). This is considered a root-based mechanism. Type II consists of the shoot tolerance to a high Fe concentration, likely through the compartmentalization via storage within the inner cavity of ferritin proteins (Stein et al. 2009) or by the action of vacuolar transporters (Zhang et al. 2012). Meanwhile in Type III, the presence of an antioxidant system that detoxifies the reactive oxygen species produced via Fenton when Fe that presents in excess (Wu et al. 2017). Types II and III are considered as shoot-based mechanisms.

Some of the genes involved in the mechanism of Fe absorption by rice plants include the *IRT* (*Iron Regulator Transporter*) transporter gene. The Fe^{2+} ions that have been absorbed will be transported by the protein that coded by *IRT* gene to different parts of the plants, through the mechanism of Fe^{2+} partitioning so that the plants can be more tolerant to the excessive Fe^{2+} conditions (Rout et al. 2015). The molecular markers that linked to the *OsIRT* gene on rice (*Oryza sativa, IRT*) have been designed and used to assist the selection process to support the breeding programs for the swamp rice lines development. These molecular markers have been used for selecting the breeding lines derived from a crossing using the multi parents varieties to develop the Fe-tolerant rice lines.

The Marker Assisted Selection (MAS) application on molecular breeding approach can improve the precision of the selection based on these superior genes. Moreover, molecular breeding can also determine the direction of selection, for example, it can minimize the linkage drag of undesired insertion. However, molecular breeding can not stand alone, but it still needs to be completed by selection based on the phenotypes. The purpose of this study was to evaluate the agronomic performance, including yield and yield characters of the promising molecular breeding, the Fe-toxicity tolerant lines in the shallow rainwater inland swamps in Sorong, West Papua, Indonesia.

MATERIALS AND METHODS

Genetic material

Fifteen advanced promising rice lines developed from various genetic backgrounds (Table 1) were used in this experiment. The evaluation in different locations and different seasons could have significant information for fluctuations in yield and yield components characters due to variations in soil fertility, unpredictable rainfall, and the presence of other biotic and abiotic stresses.

To confirm the genotype profiles, the selected lines used were analyzed using the specific primer for *IRT* alleles, *OsIRT1* (F: TCTTCCACCCTGAGCAGCTC; R: AACCTTGGAGACCAGTGCAG) (Utami et al. 2020).

Agroecosystem of the testing location

The experiment was conducted in a shallow inland swampland with a puddle height of >100 cm in Makbalaim Village, Mayamuk Sub-district, Sorong District, West Papua Province, Indonesia (Figure 1). The field experiment was conducted during planting season-II, starting from August to November 2018.

The altitude of trial location is 285 m above sea level. The climatic condition at the field site is humid tropic. The rainfall average 2836.4 mm per year with the number of days 107-185 rain. The average air temperature ranges between 25.09ºC (minimum) and 27.15ºC (maximum) with 87% air humidity. The average rainfall during experiment was 2809.25 mm per year with 210 days of rain and a wind speed of 4.17 Knots (Meteorology station Domine Edward Osok, Sorong City 2018).

Soil characteristics of testing location

The experiment was conducted in inland swamp condition with an alluvial soil type. The soil physical and chemical properties are shown in Table 2.

The soil texture was dominantly silty-clay. This soil has more fine particles so that it can hold water and nutrients better than the sandy soil type, but it can cause a lesser yield increase due to high nitrogen level (Ye et al. 2007). The organic material showed that C-organic was low while N-total was medium. The C/N ratio also was categorized into medium. A low C/N ratio condition tended to exhibit the net N mineralization, which affected the bioavailability of N mineral (Mohanty et al. 2010). In contrast, a high C/N ratio will cause the exhibition of N immobilization, which prevents the N from being accessible to plants (White 2005).

Table 1. The genetic material used in this research and their parents for crossing

| Line number	Parents crossing
G1	Kao Daok Mali-105-9 / B13143-8-MR-3-KA-14 // Inpara 5
G2	Setail / Inpara 2 // Code
G4	B11844-MR-29-7-1 / Inpara 3 // Cisantana
G5	B11844-MR-29-7-1 / Inpara 5 // Code
G6	IR42 / Ciherang
G7	Banyuasin / Ketankutuk
G8	Siakraya / B13132-7-MR-1-KA-6
G10	Swarna Sub-1 / Mekongga
G11	Swarna Sub-1 / Ciherang
G13	Batanghari / Conde
G14	Inpari 9 / Swarna Sub-1
G16	Cimelati / Inpara 3 // Inpari 9 / FR13A
G18	Mekongga / Inpara 3 // Mekongga / Inpara 3
G21	IR64 Kebo / BR11 Sub-1
G22	Ciherang / Swarna Sub-1 // Ciherang /// Inpara 3
G33 (IR64)	Negative control (sensitive to Fe-toxicity)
G34 (Mahsuri)	Positive control (tolerant to Fe-toxicity)
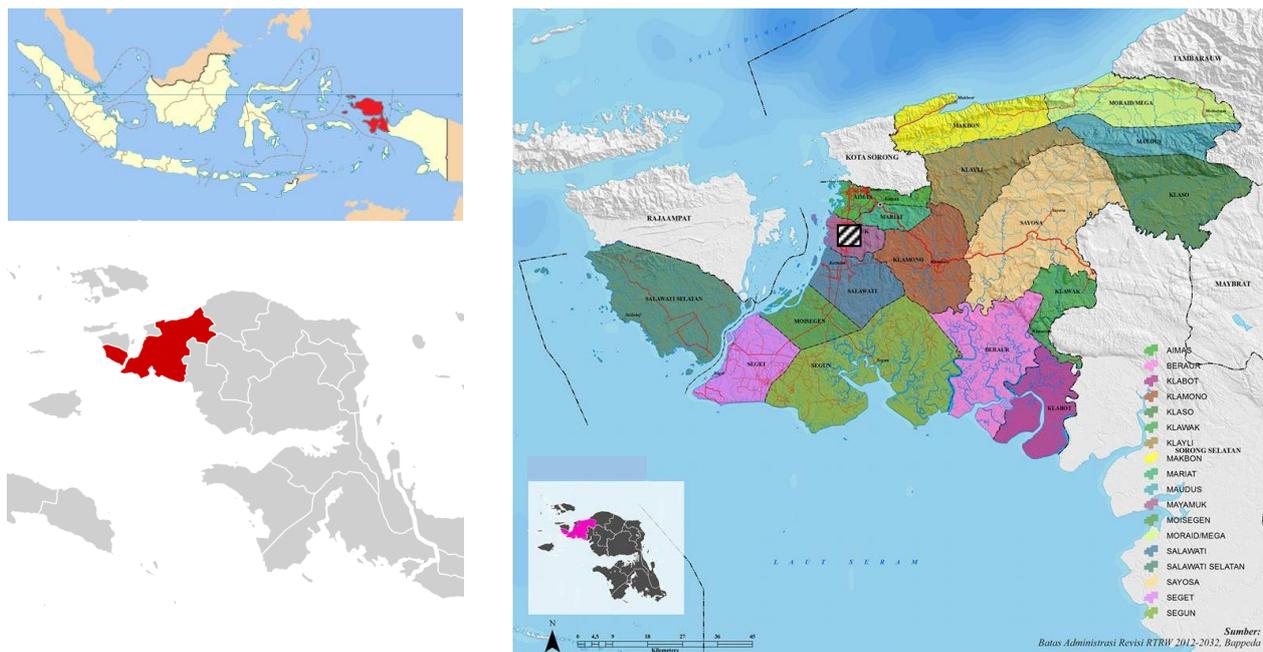
Figure 1. The map of field experiment location in Makbalaim Village (rectangle), Mayamuk Sub-district, Sorong District, West Papua Province, Indonesia

Table 2. Soil physical and chemical properties of testing location.

Soil texture (%)			Organic material %			pH H$_2$O	HCL 25% (mg/100g)		Sample soil dryness (105°C)				Fe (ppm)	Total (NHO$_3$) %		
Sand	**Silt**	**Clay**	**C org**	**N total**	**C/N**		**P$_2$O$_5$**	**K$_2$O**	**Ca**	**Mg**	**K**	**Na**		**Fe**	**S**	**Piryt**
0	45	55	2.28	0.26	19	4.6	176	11	9.75	8.45	0.13	30.35	367	7.24	0.01	0.02
1	42	58	4.28	0.23	18	4.6	139	9	9.79	8.52	1.10	0.33	368	6.60	0.01	0.02
2	41	58	3.38	0.25	17	4.7	128	8	9.93	8.47	0.10	0.35	477	7.38	0.01	0.02

Source: Soil analysis results by Soil Research Laboratory of Indonesia Soil Research Institute, Bogor, West Java, Indonesia.

Observed parameters

The experiment was conducted in a randomized block design, with 3 replications and 17 genotypes as treatments, two of them were a sensitive control variety (IR64) and a tolerant control variety (Mahsuri). The experimental unit was a plot measuring 4 m x 5 m. The planting was conducted with a spacing of 25 cm x 25 cm after the seedlings were 21 days old. The seeds were planted one seedling per hole. The fertilizers used were organic and inorganic fertilizers. The organic cow-manure was used as basic fertilizer and given after the soil was processed with a dose of 5 kg per plot (2.5 tons.ha^{-1}). An inorganic fertilizer used was NPK Phonska with a dose of 100 kg.ha^{-1}. Each fertilizer was given 2 times, first at 14 DAP (days after planting) and the second fertilizer at 30 DAP, on a half dosage. The irrigation was done intermittently for 4 wet days and 4 dry days. The weed control was conducted mechanically by using porcupines at 4 DAP (1-month-old plants) and 8 DAP (2 months old plants). Furthermore, the pest control was carried out according to the field conditions with the inorganic pesticides, Regent80 to control the main pest, like *Nilaparvata lugens* and *Scirpophaga* sp.

Scoring the bronzing symptoms for the Fe-toxicity parameters was referring to the SES method, shown in Table 3. The agronomic characters, yield component, and grain yield characters were observed according to Standard Evaluation System for Rice (IRRI 2014).

Genotype analysis
DNA isolation

DNA isolation was performed using the CTAB method according to Doyle & Doyle (1987). The plant leaves were ground in liquid nitrogen by a tissue lyser. A sample was added with 750 μL Cetyltrimethyl Ammonium Bromide (CTAB) buffer and incubated in 65°C for 30 min. This suspension was then added with 750 μL CI (chloroform: isoamyl alcohol = 24 : 1) and centrifuged at 10,000 rpm for 15 minutes. The supernatant was moved into a new tube and then added with 50 μL Na-acetate 2M pH 5.2 and 1 mL absolute ethanol and incubated overnight at -20°C. After freezing overnight, it was centrifuged at 10,000 rpm for 15 minutes. A pellet was washed with 500 μL 70% alcohol and centrifuged at 10,000 rpm for 5 minutes. A dried pellet was added with 50 μL 1×TE and 10 μL of 10 ng.μL^{-1} RNase and incubated at 37°C for one hour. The inactivation

of RNase was performed by incubation at 65°C for 15 minutes. DNA quality was tested by electrophoresis on 0.8% agarose gel in 1×TAE buffer at 100 volts for 60 minutes, then visualized using UV light (BioRad, USA). The DNA quantity was determined using the NanoDrop 2000c Spectrophotometer (Thermo Scientific, USA).

PCR analysis

PCR analysis was included in the markers selection and the amplification of DNA fragment. In the markers selection, PCR analysis was performed in a total volume of 10 μL containing 4 μL DNA (10 ng), 0.5 μL primers-F 2.5 pmol, 0.5 μL primers-R 2.5 pmol, and 5 μL of KAPA. PCR conditions were pre-PCR at 94°C for 5 minutes, denaturation at 94°C for 45 seconds, annealing at 55°C for 1 minute, elongation at 72°C for a minute, and post-PCR at 72°C for 7 minutes. PCR process was performed for 35 cycles. PCR products were analyzed using an electrophoresis on agarose gel 2% in a buffer solution of 1×TAE buffer (40 mM Tris-acetate, 1 mM EDTA) at 50 volts for 60 minutes. DNA bands in agarose were visualized with UV light. DNA fragments of selected Fe-tolerant rice (41 populations) were amplified using 14 selected primers related to rice grain quality. PCR reactions were conducted in same procedure as in markers selection, but an annealing temperature was adjusted for optimization temperature of each primer for 1 minute. DNA visualization was subsequently measured by GelQuant.NET 3.5 to determine the size of the base of each DNA band.

Data analysis

The data obtained were analyzed statistically using the analysis of variance (ANOVA) and the mean difference between each test line with the check varieties was determined using the Least Significant Increase (LSI) (Peterson 1994) at a 5% confidence level.

RESULTS AND DISCUSSION

Fe-toxicity tolerance level

The ANOVA of the Fe-toxicity tolerant characters showed a significant result (<0.001) only for the root length character. The ANOVA results revealed that all the lines tested have the same performances in the bronzing score

and biomass, but the different performance on the length of roots.

The F toxicity tolerance character's scores of the total of 15 lines, as compared to the check varieties at the vegetative stage were varied, ranged from 1 to 5, while at the generative stage, there was indication of the recovery of the Fe tolerance performance shown by the decreasing bronzing scores with a range from 1 to 3 (Table 4). There were 10 lines that had a steady tolerance level on both vegetative and generative stages, i.e. G1, G2, G4, G5, G7, G8, G10, G11, G21, and G22.

The performance of root lengths of the lines shown in Table 4 indicated that G18 and G5 had the best performance on root length, as showed on the LSI mean difference, they had close performance to the positive check variety, G34. They had the root length reached up to 27.2^{ab} cm and 25.2^{bc} cm, respectively, while 28.1^{a} cm for G34. This indicated that the tested plants could grow and develop well due to their long roots that can absorb nutrients properly. Koesrini et al. (2018) also reported that based on their observation in tidal swampland, the rice plants that intolerant to iron toxicity had a little short and dark brown roots, so the nutrient absorption was poor and had affected on a short plant height. Nevertheless, the correlation analysis showed that there was no correlation (coefficient correlation: 0.09) between bronzing score to root length.

The biomass performance of the rice lines tested showed that all lines had higher biomass than the negative control plant (Fe sensitive variety), G33. It indicated that all lines had a better performance on Fe-toxicity than G33. Nevertheless, several lines had lower biomass than the positive control plant, G34. The G5 line had the best on both root length and biomass performance, while G11 and G21 had the finest biomass performance. There was significant correlation between bronzing score to weight of biomass (coefficient correlation: -0,58**). It seems indicated that the tolerance plants (low bronzing score) have higher biomass weight. While the correlation between root length and biomass was also significant (coefficient correlation: 0.59**), so that means the root length and biomass were significant parameters for Fe tolerance but not directly correlated to bronzing score. The selected lines based on bronzing score, root length and biomass parameters were showed as the worthy promising lines since they had a good response tolerant to Fe-toxicity.

Table 3. The tolerance score of Fe-toxicity in rice plants.

Score	Plants symptoms	Tolerance level
1	Normal growth and tillers	Highly Tolerant (HT)
3	Growth and tillers are somewhat normal, dark brownish-red, purple or orange yellowish leaves	Tolerant (T)
5	Stunted growth and tillers, many leaves are reduced	Moderate Tolerant (MT)
7	Growth and tiller, generally bronzing leaves or dies	Sensitive (S)
9	All plants almost die or die	Highly Sensitive (HS)

Note: Scoring symptoms of Fe-toxicity observed at 1 DAP and 8 DAP with referring to the SES method (IRRI 2014). Score 1 = highly tolerant, 3 = tolerant, 5 = moderate tolerant, 7 = sensitive, and 9 = highly sensitive.

Table 4. The Fe-toxicity performance of rice lines tested

| Lines | Bronzing score | | Root length (cm) | Biomass (g) |
	Vegetative stage	Generative stage		
G1	1 (HT)	1 (HT)	22.4[de]	835.3
G2	3 (T)	1 (HT)	24.9[bcd]	714.2
G4	1 (HT)	1 (HT)	21.4[ef]	832.2
G5	1 (HT)	1 (HT)	25.2[bc]	865.9
G6	5 (MT)	3 (T)	19.6[fg]	779.3
G7	1 (HT)	1 (HT)	20.5[efg]	876.7
G8	1 (HT)	1 (HT)	18.6[g]	827.7
G10	1 (HT)	1 (HT)	22.8[cde]	874.0
G11	1 (HT)	1 (HT)	15.4[h]	1,403.7
G13	5 (MT)	3 (T)	22.5[de]	739.5
G14	5 (MT)	3 (T)	19.7[fg]	719.2
G16	3-5 (MT)	3 (T)	20.9[efg]	690.6
G18	5 (MT)	3 (T)	27.2[ab]	683.1
G21	3 (T)	3 (T)	19.9[fg]	844.6
G22	1-3 (T)	1 (HT)	24.1[cd]	819.2
G33	5 (MT)	5 (MT)	22.7[cde]	558.5
G34	1-3 (T)	1 (HT)	28.1[a]	834.3

Note: HT = highly tolerant; T = tolerant; MT = moderate tolerant

During Fe-toxicity, the direct effect was shown on the tiny brown spots which appear on the leaf tips and spread to the leaf base, and then will result in the reddish-colored leaves followed by the drying of leaves, this condition is known as a leaf bronzing process and most recognized as the morphological symptom of Fe-toxicity (Becker and Asch 2005). The leaf bronzing typically appears first in the older leaves with higher transpiration rates (Aung et al. 2018). Besides the leaf performance, the Fe-toxicity stress will also affect the root and shoot performances (Stein et al. 2019). The leaf bronzing, root length, and weight of biomass were used as the parameters of the rice lines tolerance to Fe-toxicity.

Agronomic characters and yield components

The ANOVA showed the significant (<0.001) effect of rice genotypes on all yield component characters, except the total number of grain per panicle. The ANOVA results revealed that all the lines tested gave different performances on agronomic and yield component characters. These results also indicated that this experiment in inland swamp could be screening the Fe toxicity tolerance performance of rice lines tested. So, it could be also possible for selecting the best performance lines on agronomy and yield component characters.

The ANOVA results showed that all characters among all tested rice were significantly (P<0.01) different, except on a total amount of grain per panicle. This result became obvious in a high CV (%) for a total number of grains per panicle (10.22%), which contained two characters, a number of filled grain (13.96%) and a number of empty grain (36.94%), which was the highest for all examined traits. The coefficients of variation (CV) are a precision measure in experiments that been done.

Further, the results of testing for all lines evaluated showed that there were diverse performances on some

characters (Table 5). The plant height performance dominated on >60 cm, >80 cm, and <120 cm at 4, 6, and 8 WAP, respectively. All the lines tested had a date of maturity less than 100 days, except for G21 and G22. Meanwhile, for a yield component, the G6 line had the longest panicle (PL) which reached 25.9 cm. The G8 line had the biggest number of filled grain (N-FG), while the G6 line had the smallest number of empty grain (N-EG) and the G11 line had the biggest number of total grain (N-TG) per panicle.

Grain yield evaluation

The ANOVA results of grain yield characters showed a significant (<0.001) different on all rice tested. Those ANOVA results revealed that all the lines tested gave different performances on the thousand-grain weight and yield characters. These results indicated that an inland swamp that used as the evaluation site gives a different selection effect to the lines tested, so that it could be possible to select the lines which had the best performance on agronomic and yield component characters. Table 8 showed that the performance of grain weight and yield characters have a significantly different (P<0.01) among all the lines tested.

The post hoc test showed significantly different performances of tested rice lines on some characters (Table 6). The best line for thousand-grain weight was G11, which produced 21.40 grams. Meanwhile, for a total yield per plot, the line of G1 showed the best performance which gave a total of 12.30 kg/per plot or could be converted as 6.2 ton.ha^{-1}, and the second next better-selected line was G7 which had a yield of 11.83 kg/per plot or could be converted as 5.9 ton.ha^{-1}.

The experiment was conducted in an inland swamp area which categorized as non-tidal swamp. This area is a river flood plain that receives no effect from the sea tides (Irawati et al. 2015). The main problem of an inland swamp is water puddle, while the soil fertility is not dominant in affecting the rice cultivation (Noor 2007). In general, the level of soil fertility in an inland swamp is higher than in a tidal land. In puddle condition, the Fe-toxicity often emerges and inhibits the plant growth, this condition causes the rice yield to decline by up to 90% in a high Fe level in the Yellow Red Podsolik soil type in Lampung, Sumatra (Suhartini 2004).

During a rainy season, non-tidal swamp areas are flooded by the river and the floodwater doesn't recede until dry season. The water volume in non-tidal swamp significantly will depend on rainfall; it is deeper in rainy season and will gradually decrease during dry season. Therefore, the planting usually will start at the end of rainy season or depend on the water level in the field. The main problem faced by the farmers was due to the unstable water level and unpredicted weather. The common situation in the field was high flooding prior to planting and extreme drought during the reproductive stage. This situation had caused the farmers need to adjust their planting time and to reconsider the use of proper cultivar combined with some improved cultivation techniques (Sulaiman et al. 2019).

Table 5. The results of LSI test for further analysis of agronomic and yield component characters on the rice lines tested

Lines	Plant Height (cm)			DF	DM	PL	N-FG	N-EG	N-TG
	4 WAP	6 WAP	8 WAP						
G1	57.11[a]	81.44	110.55[a]	75[ab]	90[fg]	27.23[ab]	163.80[ab]	36.40[ab]	200.2[efg]
G2	50.74	72.67	106.55	75[ab]	85[h]	26.70[bcd]	169.80[ab]	36.80[ab]	204.3[g]
G4	60.41[a]	84.22	118.55[a]	70[ab]	87[gh]	26.77[bc]	157.67[ab]	50.40[a]	207.9[bcd]
G5	62.04[a]	85.78[a]	116.15[a]	72[ab]	87[gh]	26.76[bc]	157.73[ab]	60.40[a]	221.5[efg]
G6	61.52[a]	84.74	113.15[a]	62[ab]	81[i]	25.97[cde]	207.67[a]	24.00[ab]	231.4[bc]
G7	60.07[a]	82.78	116.37[a]	66[ab]	80[ij]	26.15[cd]	212.27[a]	25.60[ab]	240.07[a]
G8	61.29[a]	83.63	111.82[a]	65[ab]	95[de]	25.99[cde]	218.00[a]	25.73[ab]	243.8[cdefg]
G10	61.59[a]	87.26[a]	117.59[a]	80[b]	94[de]	26.96[bc]	199.60[a]	30.40[ab]	236.3[ab]
G11	64.44[a]	90.07[a]	130.19[a]	79[b]	94[de]	27.26[ab]	207.90[a]	64.00[a]	270.7[cdef]
G13	60.63[a]	90.11[a]	120.37[a]	79[b]	96[d]	25.67[de]	179.87[ab]	47.13[a]	227.53[fg]
G14	60.22[a]	86.63[a]	117.44[a]	81[b]	92[ef]	26.24[bcd]	150.53[ab]	66.80[a]	218.8[defg]
G16	62.18[a]	85.30[a]	120.82[a]	77[tn]	100[c]	26.20[bcd]	175.90[ab]	67.80[a]	243.3[cdef]
G18	63.04[a]	88.33[a]	118.44[a]	85[ab]	95[de]	25.11[e]	163.90[ab]	65.33[a]	235.6[efg]
G21	63.45[a]	93.15[a]	133.67[a]	80[b]	104[a]	26.46[bcd]	180.10[ab]	79.07[a]	250.3[fg]
G22	39.48[tn]	75.70	114.03[a]	89[b]	104[a]	25.06[e]	157.90[ab]	69.20[a]	234.3[cdef]
G33[(a)]	53.63	84.82	110.07	79	87[gh]	24.04[f]	114.93[b]	90.93	214.9[cdefg]
G34[(b)]	66.11	93.26	136.89	94	104[a]	27.97[a]	169.07[ab]	43.07	208.1[bcde]

Note: WAP = Week After Planting, DF = date to flowering, DM = date to maturity, PL = panicle length, N-FG = number filled grain per panicle, N-EG = number empty grains per panicle, N-TG = total amount of grain per panicle

Table 6. The post hoc LSI test for thousand-grain weight (TGW) and yield characters of the rice lines tested

Lines	TGW (g)	Yield	
		(kg/plot)	(ton.ha^{-1})
G1	18.97[efg]	12.30[ab]	6.15
G2	17.43[g]	7.07[ab]	3.53
G4	22.33[bcd]	8.53[ab]	4.27
G5	19.07[efg]	8.67[ab]	4.33
G6	22.60[bc]	6.43[ab]	3.22
G7	26.47[a]	11.83[ab]	5.92
G8	19.53[cdefg]	10.80[ab]	5.40
G10	24.43[ab]	10.97[ab]	5.48
G11	21.40[cdef]	10.27[ab]	5.13
G13	18.57[fg]	5.97[b]	2.98
G14	19.20[defg]	6.37[ab]	3.18
G16	20.73[cdef]	5.27[b]	2.63
G18	19.03[efg]	6.73[ab]	3.37
G21	18.23[fg]	7.73[ab]	3.87
G22	20.83[cdef]	10.60[ab]	5.30
G33[(a)]	19.73[cdefg]	5.87	2.93
G34[(b)]	21.87[bcde]	3.03	2.05

Based on the results of field experiment, it showed that there were variations of Fe-toxicity tolerance responses on the rice lines tested. There was a wide variation in tolerance, which depended on the stress duration, strength, and plant development stage. Some genotypes might show the contrast performances depending on how and the location of experiments accomplished. The root is a part of plant which interacted directly with the soil performance, including the toxic Fe^{2+} level contained in the soil. The exposure of Fe^{2+} excess affected roots and continued to severe reduction of chlorophyll concentration in the leaf as will be shown as the bronzing spot. Nevertheless, there was no reduction in the tolerant one (Stein et al. 2019).

The previous research showed that (1) photosynthesis is affected by Fe-toxicity (Müller et al. 2017); it is known that the Fe uptake genes are down-regulated upon a high Fe treatment (Finatto et al. 2015); and that early (3 days) and late (3 weeks) Fe-toxicity responses are quite different in both roots and shoots (Quinet et al. 2012). However, the molecular mechanisms associated with tolerant and sensitive genotypes are a lot of underexplored. The lines evaluated are known contained the *OsIRT* allele gene which plays a role in regulating Fe uptake and transport in the genome of rice plants so that plants are tolerant. Nugraha and Rumanti (2017) confirmed the availability of rice plants giving a good production in a swampland with the iron toxicity condition depends on the condition of environment and the mechanism of plants in absorbing iron. One of the Fe-toxicity effects can be seen when the rice plant entering the end of the vegetative stage or the initial generative stage. The Fe-toxicity inhibits the formation of panicles and also the number of grains in each panicle (Singh et al. 1992). The Fe-toxicity also causes the plants to be sterile or disrupt the flowering (Virmani 1977). Virmani (1977) and Suhartini (2004) also reported that a decrease in the rice production was caused by iron toxicity, especially in an inland swamp, such as in Taman Bogo, Lampung, South Sumatera reached up to 70% loss yield production for intolerant varieties, while for tolerant varieties reached up to 30%. So, the tolerant rice varieties that adaptive in a specific swamp land condition become one of important technology components to overcome the problems in the swampland agroecosystem (Kustianto 2009).

Other parameters of the Fe-toxicity tolerant are the performance of root and the biomass of plant, since their performances indicated the ability of plant to regulate the toxic effect of Fe^{2+}. As examined on the material genetic used, all the lines used in this experiment have selected by

using the *OsIRT1* marker gene. This gene plays a role in strategy I of the Fe tolerance mechanisms (Bennett et al. 2011; Rout et al. 2015), which transport Fe^{2+} from the root epidermal tissue runs through the plasma membrane into the cytosol (Rout et al. 2015). Vert et al. (2001) mentioned that the *IRT1* gene contributes to the partitioning of Fe^{2+} mechanism to several parts of plant, from root to other parts of plant so that the plants can be more tolerant in the Fe^{2+} excess condition.

The rice varieties for a swampy area are generally tall and grow rapidly, have strong culms and medium growth duration, and tolerant to abiotic stresses such as soil acidity, Fe-toxicity, and salinity (Harahap and Silitonga 1998). The plant height is one of the criteria for the selection of rice plants, but it indirectly related to the yield. The plant height has a correlation of negative direct effect on grain yield but positive indirect effect through a number of productive tillers per hill (Hairmansis et al. 2010). Based on the observation of rice lines tested during the generative stage, especially when the panicles are formed, the lines tested start to be attacked by the stem borer pest. The impact of this condition, it causes the seed filling process to be hampered due to the damage to the plant stem so that it produces un-hulled rice. The empty grain will effect the rice yield. This is as reported by Ariati et al. (2016), stating that a higher percentage number of empty grain affected a lower rice yield. The empty grain shows the inability of plants to fill the grains of plants. The agronomic and yield components evaluation showed that G8 has the highest number of filled grain, with the plant height of 111.82 cm, the days to flowering of 65 days, and the days to maturity of 95 days.

The evaluation of grain yield showed that G1 line has the highest yield (6.15 t.ha^{-1}) but not for TGW character. This is related to Harmansis et al. (2010) stated that TGW rarely affected the grain yield. The line of G1 showed the longest panicle length (PL) comparing all the lines tested. The panicle length and grain yield showed a positive association to the Indica inbred ecotypes (Li et al. 2019). The second rank on the yield characters was G7 line that has a yield of 5.92 t.ha^{-1}. This line has the days to flowering (DF) and the days to maturity (DM) shorter than G1 line. Both G1 and G7 lines are included as Indica inbreds, since derived from the Indica parents. The performance of field experiments, G1, and G7 lines are shown in Figure 2. The two of the selected lines, G1 and G7, probably could adapt to the unstable water fluctuation in the inland or non-tidal conditions, as a major problem are high flooding in rainy season and drought in dry season.

The development of swamp rice cultivation would be beneficial to support the national rice sufficiency in Indonesia. However, many challenging constraints, both on farm and off farm, in the promising rice lines tested at an inland swamp in Sorong, West Papua revealed that the most of lines show a steady tolerant, both on vegetative and generative stages, based on bronzing, root length and biomass characters. The agronomic and yield components evaluation showed that G7 line has the highest number of filled grain, while G1 line has the highest yield and panicle length characters. The selected lines would be valuable as the candidates of swampy rice varieties, especially for an inland swamp area.

Genotype performances

The genotype performance of the rice lines detected using the specific primer linked to the *OsIRT* gene showed that the allelic variation ranged from 200 bp to 300 bp (Figure 3). The lowest size allele indicated the medium tolerance (MT) and tolerance (T), while the upper size alleles seem closely linked to Fe-sensitive/susceptible trait.

Figure 2. The performance of yield observation experiments in an inland swampy area in Sorong, West Papua. The G1 dan G7 lines are the selected lines that have high yield characters
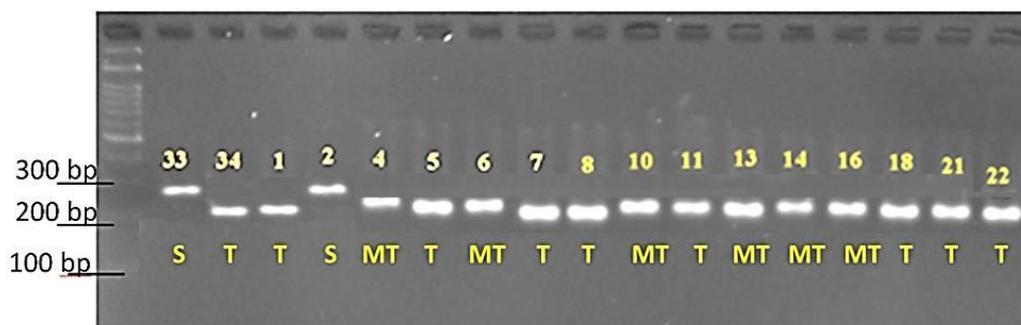
Figure 3. The genotypes performance of the rice lines using the specific marker for *IRT* gene allele. The allele size was determined using DNA ladder 100 bp marker. Note: S = Sensitive, MT = Medium Tolerance, T = Tolerance

As described before, one of the Fe-toxicity tolerance mechanisms in rice plants was contributed by the *IRT* (*Iron Regulator Transporter*) transporter gene. The Fe^{2+} ions that been absorbed will be transported by the *IRT* gene to the different parts of the plants, through the mechanism of Fe^{2+} partitioning so that the plants can be more tolerant to the excessive Fe^{2+} conditions (Rout et al. 2015). Based on this thoughtful, the tolerance plants will show a low Fe score and have a better on their shoot performance than on the root length. Due to the better root length more indicated having an inclusion regulation on the root level than partitioning mechanism. G1 and G7 are the selected lines that have the better on the shoot than their root length performances. The weight biomass of G1 line (835.3 g) was higher than the tolerance control plant, Mahsuri (G34) (834.3 g) (Table 5). The G1 line also has a good performance on panicle length (27.23 cm) closely comparable to the tolerance control plant (Table 5). The genotype performance of G1 and G7 lines showed that they have the lowest size alleles of *OsIRT* gene (Figure 3). The polymorphism performance between the tolerance and sensitive rice lines obtained by using this marker could be applied for assisting selection. Certainly, this approach has to be supported by the complete phenotype characters related to the target selection. So the associated test between genotype and phenotype characters target could be accomplished.

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