Development of High-Yielding Rice Varieties Suitable for Swampy Lands in Indonesia

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ABSTRACT Productivity of swampy land rice varieties in Indonesia remains low due to abiotic and biotic stresses. Iron toxicity, submergence and long-term flooding are common problems that affect plant growth, sometimes leading to total crop loss. Meanwhile, rice blast, rice tungro virus (RTV), and bacterial leaf blight (BLB) can also lead to severe yield loss. These stresses could be overcome by an integrative approach of cultural practices and planting multi-stress tolerant rice. Here we describe the development of new high-yielding varieties that are adapted to the swampy land environments in Indonesia with multi-stress tolerance to diverse abiotic and biotic stresses. Multi-environmental trials were performed in nine locations to screen for several abiotic and biotic stresses in the field and greenhouse in 2012 and 2013. This study identified promising rice lines that had high grain yield as well as tolerance to iron toxicity and submergence, resistance to BLB and RTV, and good grain quality. The best line was B11377F-MR-34-2, derived from a double cross among Cinglonik, IRBB7, Mamberamo and IR64. It was confirmed that this advanced breeding line carries the submergence tolerance gene, *SUB1*, through expression analysis of the *SUB1A* gene. Another promising line was B11586F-MR-11-2-2, having iron toxicity tolerance, resistance to RTV, and high milling recovery. Moreover, these lines have passed the Indonesian national variety release committee and have been named as Inpara8 and Inpara9, and are targeted for dissemination and adoption in the swampy areas.

Keywords Bacterial leaf blight, Iron toxicity, Rice Tungro virus, Submergence, Swampy land, Multi-environmental trials

INTRODUCTION

Swampy land is one of the potential areas for future development and expansion of rice cultivation. Swampy areas in Indonesia are spread across four major islands, Sumatra, Kalimantan, Sulawesi, and Papua, reaching 33.4 million ha, including 20.1 million ha of tidal swamp and 13.3 million ha of freshwater swamp (Alihamasyah 2004). However, less than 1 million ha has been used for rice cultivation. Moreover, the productivity of rice in these areas was low, with only 2-4 t/ha (Hairmansis *et al.* 2013; Nugraha *et al.* 2016), compared to the national productivity of 5.1 t/ha (http://www.bps.go.id/tnmn_pgn.php).

Increasing the productivity of swampy rice is one of the key targets to meet the growing national demand for rice in Indonesia. However, an integrated effort needs to be deployed to apply the right technologies to overcome enormous challenges in the swampy areas, including abiotic and biotic stresses.

Varying soil conditions and dynamic changes in water depth due to the changes of tidal sea level impose multiple abiotic stresses on rice crops. These problems are largely associated with water availability and quality, such as flash floods, stagnant water, drought, and salinity, as well as the nutrient status of soils, including nutrient deficiencies and toxicities, and low pH (Alihamasyah *et al.* 2003). On the

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other hand, biotic stresses such as rice pests and diseases can also significantly challenge the production of rice in these areas. The important pests in swampy areas are rats, stem borer, *Leptispa* beetle, rice hispa, armyworms, grasshopper, mole cricket, black bug, brown planthopper (BPH), green leafhopper (GLH), and stink bug. While the common diseases include leaf and neck blast, rice tungro virus (RTV), bacterial leaf blight (BLB), brown spots, and leaf scald (*Rhynchosporium oryzae*) (Asikin 2015). These various stresses fluctuate seasonally and can adversely affect rice growth and productivity.

Physiological and agronomic traits related to tolerance to various abiotic stresses that may affect rice crops in swampy areas have been previously investigated (Sahrawat 2005; Castillo *et al.* 2007; Singh *et al.* 2009; Henry *et al.* 2012; Kato *et al.* 2014). Likewise, quantitative traits loci and genes for tolerance to various abiotic stresses, including drought, salinity, submergence during vegetative stage, submergence during germination, stagnant flooding and iron toxicity have been identified and used in molecular breeding programs (Ismail *et al.* 2007; Thomson *et al.* 2010; Iftekharruddaula *et al.* 2011; Septiningsih *et al.* 2012; Septiningsih *et al.* 2013b; Baltazar *et al.* 2014; Dixit *et al.* 2014; Kretschmar *et al.* 2015; Toledo *et al.* 2015; Gonzaga *et al.* 2016; Iftekharruddaula *et al.* 2016a; Singh *et al.* 2016). In addition, elite breeding lines tolerant to multiple abiotic stresses have been developed through conventional breeding (Gregorio *et al.* 2002; Kumar *et al.* 2008; Collard *et al.* 2013a; Collard *et al.* 2013b). Allele mining to tap new genes and novel beneficial alleles from diverse germplasm for abiotic stresses have also been explored (Fukao *et al.* 2009; Li *et al.* 2011; Niroula *et al.* 2012; Iftekharruddaula *et al.* 2016b; Shah *et al.* 2016). The improved breeding lines developed from both conventional and molecular breeding and the available DNA markers can be used to develop new tolerant lines with multi-abiotic stress tolerance in more challenging areas, such as in the swampy lands.

Developing improved breeding material tolerant to most of these abiotic and biotic stresses coupled with good management practices can significantly reduce the crop damage and yield reduction. Utilization of high-yielding rice varieties tolerant to the prevalent stresses involves no

additional costs to farmers and can enhance and stabilize productivity in both rainfed and irrigated ecosystems (Ismail and Tuong 2009; Thomson *et al.* 2009; Septiningsih *et al.* 2013a). The key traits needed for successfully growing rice in the swampy areas include improvement of yield potential, tolerance to abiotic stresses such as submergence, stagnant flooding, salinity, and Fe and Al toxicity, resistance to major pests and diseases including BPH, blast, BLB, and tungro, as well as improving the grain quality. Here we reported the development of high-yielding improved lines having tolerance to multiple abiotic and biotic stresses and good grain quality, which show promise to increase rice production in the swampy lands in Indonesia.

MATERIALS AND METHODS

Plant material

Nine promising swampy rice breeding lines, namely: IR84941-12-1-2, IR83835-95-1-1-3, B11377F-MR-34-2, B11586F-MR-11-2-2, B10891B-MR-3-KN-4-1-1-MR-1, BMIP-46-4-1, AGH42-2-3, AGH43-1-2, GH137-7-SKI-B, were used in this study along with three check varieties, IR42, Inpara2, and Martapura. Several other varieties were also used as specific checks, depending on the type of abiotic or biotic screening test being performed. IR84941-12-1-2 and IR83835-95-1-1-3 were originally from the International Rice Research Institute (IRRI), Los Baños, Philippines, but have undergone selection at the Indonesian Center for Rice Research (ICRR), Sukamandi. B11377F-MR-34-2, B11586F-MR-11-2-2, and B10891B-MR-3-KN-4-1-1-MR-1 were internally bred in ICRR. BMIP-46-4-1 was from the Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD), Bogor. The last three breeding lines were obtained from Bogor Agriculture University (BAU).

Submergence screening

Testing of swampy rice material to submergence tolerance during vegetative stage was conducted on an artificial bed at ICRR, Sukamandi, as described by

Septiningsih *et al.* (2015). An additional screening for a subset of the germplasm was also performed at the screen house at IRRI, Los Baños, Philippines. Data from IRRI was analyzed using PBTtools. IR42 and FR13A were used as the susceptible and the tolerant checks, respectively. Plants were completely submerged under 1 m water at 14 days after transplanting. Water was drained at 14 days after submergence, when IR42 plants were mostly dead. The survival rate was counted seven days after desubmergence.

SUB1A allele identification and expression analysis

The *SUB1*-specific markers GnS2, RM8300 and ART5 were used to further investigate the submergence tolerant line identified based on phenotyping (Neeraja *et al.* 2007; Septiningsih *et al.* 2009; Septiningsih *et al.* 2013a). DNA from one week-old seedlings was extracted following a standard protocol (Zheng *et al.* 1995). Total RNA was isolated using TRIzol (Gibco) from 14d-old submerged seedlings as described in Xu *et al.* (2006). The SUB1A203 primer was used for the reverse transcription polymerase chain reaction (RT-PCR) (Septiningsih *et al.* 2009). PCR and RT-PCR analysis were performed as described by Xu *et al.* (2006).

Screening of Fe toxicity

Screening for Fe toxicity was conducted in the acid sulfate field in Karang Agung, South Sumatra. The experimental design was check stripe, with a tolerant check Mahsuri and a sensitive check IR64. Screening of iron toxicity was conducted in the field under an average total iron (Fe^{2+}) concentration of 592 ppm. Twenty-one-day-old rice seedlings were transplanted with one seedling per hill. Each entry was planted in a plot of 50×500 cm, with a spacing of 25×25 cm. Scoring of Fe toxicity symptoms was performed at the vegetative stage based on the Standard Evaluation System (SES) for Rice from IRRI and INGER (2014) (Supplementary Table 1).

Screening of blast disease

Screening for blast disease was performed at the seedling stage. *Pyricularia grisea* isolates were propagated on potato dextrose agar for 5-7 days, then transferred to sporulation media of oatmeal agar for 12 days, with density

of spores of $2 \times 10^5/\text{ml}$. Inoculation was performed at 18-21 days after sowing. Inoculated plants were incubated in a humid room for 48 hours, and then were moved to a screen house with humidity over 90% at ICRR, Sukamandi. Scoring was performed at seven days after inoculation based on the SES scale from IRRI and INGER (2014) (Supplementary Table 2).

Screening of bacterial leaf blight

Artificial inoculation of BLB pathotype IV and VIII was performed following the clipping method at 55-60 days after sowing. Inoculum was developed from *Xanthomonas oryzae* culture grown on Wakimoto media. Scoring of the symptoms was performed at two and three weeks after inoculation based on the SES from IRRI and INGER (2014) (Supplementary Table 3).

Screening of tungro virus

Screening for tolerance to tungro virus was performed artificially. Tungro virus was transmitted by GLH (*Nephotettix virescens*) at ten days after planting. Screening was carried out in two phases. The first phase was a mass screening and the second phase was a test tube experiment to avoid escape. This was done by forcing the insect to bite the infected leaf and facilitating virus transfer from leaf to insect (due to no feeding choice) for a period of 24 hours. The inoculums were from Garut and Subang, West Java. Scoring of the symptom was performed at seedling stage, based on the SES scale from IRRI and INGER (2014) (Supplementary Table 4).

A disease index (DI) for the genotypes can be calculated as follows:

$$\text{DI} = \frac{n(1) + n(3) + n(5) + n(7) + n(9)}{tn}$$

Note: n=number of plants showing a reaction in the scale 1, 3, 5, 7, or 9

tn=total number of plant scored

On the basis of the DI, the genotypes can be classified as resistant/tolerant: 0-3, moderate: 4-6, or susceptible: 7-9.

Multi-environment trials

Multi-environment trials were performed in the

following locations and seasons: (1) Subdistrict Kayu Agung, District Ogan Komering Ilir, South Sumatra, in dry season (DS) 2012 (fresh water swamp); (2) Subdistrict Karang Agung, District Banyuasin, South Sumatra, in wet season (WS) 2013 (tidal swamp); (3) Subdistrict Karang Agung, District Banyuasin, South Sumatra, in DS 2013 (tidal swamp); (4) Subdistrict Karang Agung, District Banyuasin, South Sumatra, in DS 2013 (tidal swamp with acid sulphate soil toxicity); (5) Subdistrict Karang Agung, District Banyuasin, South Sumatra, in WS 2013 (tidal swamp with acid sulphate soil toxicity); (6) Subdistrict Barabai, District Barito Koala, South Kalimantan, in DS 2013 (tidal swamp); (7) Subdistrict Banjar, South Kalimantan, in DS 2013 (tidal swamp); (8) Subdistrict Ketapang, West Kalimantan, in DS 2013 (tidal swamp); and (9) Subdistrict Kayu Agung, District Ogan Komering Ilir, South Sumatra, in DS 2012 (fresh water swamp). Twelve entries were used, including the nine promising lines and the three popular varieties as checks (IR42, Inpara2, and Martapura). Each rice entry was planted in a plot of 4×5 m, following randomized complete block design (RCBD) with three replications. Twenty-one-day-old seedlings were transplanted with 25×25 cm spacing and 1-2 seedlings per hill. All the recommended rice cultural practices for growing rice were applied. Grain yield per plot (kg/plot) was measured and used to estimate

yield per hectare. The pooled analysis of variance was used to analyze genotype×environment (G×E) interaction and the stability of the genotypes across environments. The stability of yield performance for each rice genotype was calculated using regression (Finlay and Wilkinson 1963) and additive main effects and multiplicative interaction (AMMI) model analysis (Gauch *et al.* 2008). A stability model was analyzed using methods of Eberhart and Russell (1966).

RESULTS

Response to abiotic stresses

Eight promising lines, except for IR83835-95-1-1-3, were highly tolerant to Fe toxicity; while eight lines were susceptible to complete submergence during vegetative stage, with the exception of one line, B11377F-MR-34-2 (Table 1). The survival rate of this line was comparable to those of FR13A and IR64-Sub1. The tolerance allele of B11377F-MR-34-2 to submergence was assayed using three *SUB1* markers, GnS2, ART5 and SC3 (Fig. 1). Interestingly, the alleles of B11377F-MR-34-2 in all three markers were the same as the allele of the susceptible check, IR42. However, upon expression analysis study of the *SUB1A* gene, the gene expression of *SUB1A* in

Table 1. Response of promising rice lines to submergence and Fe toxicity stresses.

No.	Promising lines/check varieties	Submergence (14 days) ²⁾	Fe toxicity ²⁾
1	IR84941-12-1-2	S	T
2	IR83835-95-1-1-3	S	MS
3	BMIP-46-4-1	S	T
4	AGH42-2-3	S	T
5	AGH43-1-2	S	T
6	GH137-7-SKI-B	S	T
7	B11377F-MR-34-2	T	T
8	B11586F-MR-11-2-2	S	T
9	B10891B-MR-3-KN-4-1-1-MR-1	S	T
10	IR42 (Check for S to submergence)	S	MS
11	Inpara2	S	MT
12	Martapura	S	T
	Mahsuri (check for T to Fe toxicity)	-	T
	IR64 (check for S to Fe toxicity)	-	S
	FR13A (check for T to submergence)	T	-

²⁾S: susceptible, T: tolerant, MS: moderately susceptible, MT: moderately tolerant.

Fig. 1. Different alleles of GnS2, ART5 and RM8300 in the *SUB1* gene region.

B11377F-MR-34-2 was as strong as those of in the submergence tolerant parents, FR13A and IR64-Sub1 (Fig. 2). A susceptible Indonesian variety, Padi Cina, was used in this analysis and both allele assessment with the three DNA markers and *SUB1A* gene expression confirmed its susceptibility. An Indonesian tolerant landrace, Untup Rajeb was also investigated and based on allele assessment and *SUB1A* gene expression analysis, the submergence tolerance of this variety comes from the *SUB1A* gene. Independent screening of submergence tolerance in a controlled submergence tank in IRRI confirmed the tolerance of B11377F-MR-34-2 and Untup Rajeb (Supplementary Table 5).

Response to biotic stresses

Blast

Entries showed different responses to inoculation of four predominant blast races in Indonesia (Table 2). B11377F-MR-34-2 was moderately resistant to blast race 133, and moderately susceptible to races 073 and 173. This was similar to the IR42 response and a better response than in Inpara2. Martapura was moderately resistant to race 033 and 133, but susceptible to the other two races. The most resistant line, BMIP-46-4-1, was resistant to race 033, moderately resistant to race 133 and 173, and moderately susceptible to race 073. The most susceptible lines were IR84941-12-1-2 and IR83835-95-1-1-3.

Bacterial leaf blight

The elite lines had different reactions to the three pathotypes of BLB during the vegetative stage (Table 3). Six of the elite lines had better resistance to BLB compared to the three checks (IR42, Inpara2 and Martapura).

Fig. 2. Reverse transcription polymerase chain reaction of *SUB1A* (top) with *Tubulin* as control (bottom) shown below each sample. Total RNA were isolated from leaves of 14-d-old seedlings submerged for 6 hours (S) and non-submerged controls (C).

Table 2. Reaction of elite swampy rice genotypes to four blast disease isolates.

No.	Lines/varieties	Reaction to blast diseases ²⁾			
		Race 033	Race 073	Race 133	Race 173
1	IR84941-12-1-2	S	S	S	S
2	IR83835-95-1-1-3	S	S	S	S
3	BMIP-46-4-1	R	MS	MR	MR
4	AGH42-2-3	S	S	MS	MS
5	AGH43-1-2	S	S	MS	S
6	GH137-7-SKI-B	S	MS	S	MS
7	B11377F-MR-34-2	S	MS	MR	MS
8	B11586F-MR-11-2-2	S	S	MS	S
9	B10891B-MR-3-KN-4-1-1-MR-1	MR	S	S	S
10	IR42	MS	MS	MR	MS
11	Inpara2	MS	MS	MS	MS
12	Martapura	MR	S	MR	S
	Kencana Bali (S check)	S	S	S	S
	Asahan (R check)	R	R	R	R

²⁾S: susceptible, R: resistant, MR: moderately resistant, MS: moderately susceptible.

Table 3. Reaction of elite swampy rice genotypes to three BLB pathotypes.

No.	Lines/varieties	Reaction to BLB ²⁾		
		Pathotype III	Pathotype IV	Pathotype VIII
1	IR84941-12-1-2	MR	MS	MS
2	IR83835-95-1-1-3	R	MS	MS
3	BMIP-46-4-1	R	MS	MS
4	AGH42-2-3	MR	MS	MS
5	AGH43-1-2	R	MS	MS
6	GH137-7-SKI-B	R	MS	MS
7	B11377F-MR-34-2	R	MR	MR
8	B11586F-MR-11-2-2	R	S	MS
9	B10891B-MR-3-KN-4-1-1-MR-1	MR	MS	MS
10	IR42	MR	MS	MS
11	Inpara2	MR	MS	MS
12	Martapura	MR	MS	MS
	IR64 (S check)	MS	S	S
	IRBB5 (R check)	MR	MR	MR
	IRBB7 (R check)	R	R	R

²⁾BLB: bacterial leaf blight, MR: moderately resistant, R: resistant, MS: moderately susceptible, S: susceptible.

B11377F-MR-34-2 showed better resistance to BLB disease than IRBB5; it was resistant to pathotype III and moderately resistant to pathotype IV and VIII. This line always showed resistance to BLB across locations during multi-location yield trials (data not shown). The rest of the elite lines were moderately resistant or resistant to BLB pathotype III; but moderately susceptible to pathotype VIII and moderately susceptible or susceptible to pathotype IV.

IR42, Inpara2 and Martapura had similar reactions in which all of them were moderately resistant to pathotype III, and moderately susceptible to pathotypes IV and VIII.

Tungro virus

In the screening to tungro virus, seven elite lines, including B11377F-MR-34-2 were susceptible to two most virulent tungro virus strains in Indonesia, the Purwakarta and Garut inoculums (Table 4). Similar reactions were

Table 4. Reaction of elite swampy rice genotypes to RTV.

No.	Lines/varieties	Reaction to RTV ²⁾	
		Inoculum Garut	Inoculum Purwakarta
1	IR84941-12-1-2	S	S
2	IR83835-95-1-1-3	S	S
3	BMIP-46-4-1	S	S
4	AGH42-2-3	S	S
5	AGH43-1-2	S	S
6	GH137-7-SKI-B	S	S
7	B11377F-MR-34-2	S	S
8	B11586F-MR-11-2-2	R	R
9	B10891B-MR-3-KN-4-1-1-MR-1	MR	MR
10	IR42	S	S
11	Inpara2	S	S
12	Martapura	S	S
	IR64 (S check)	S	S
	Tukad Petanu (R check)	R	R

²⁾RTV: rice tungro virus, S: Susceptible, R: Resistant, MR: moderately resistant.

observed in all local checks, IR42, Inpara2 and Martapura. However, line B11586F-MR-11-2-2 showed highly resistance to both inoculums, which was comparable to the resistant check, Tukad Petanu. Another elite line, B10891B-MR-3-KN-4-1-1-MR-1, was moderately tolerant to both inoculums.

Adaptability of swampy rice lines

Based on stability parameters, GH137-7-SKI-B had stable yield because the regression coefficient (b_i) was close to unity (1.04) and the deviation from regression (S^2_{di}) was 0.22; however, it did not have the highest yield. B11586F-MR-11-2-2 was categorized as having stable yield as well because it had high average yield (4.16 t/ha), regression coefficient close to unity (1.02), and the relatively small deviation of 0.58 from regression. On the other hand, B11377F-MR-34-2 had a b_i value of 0.67 but had the highest average yield. It was confirmed by AMMI2 biplot that B11586F-MR-11-2-2 (line no. 8) was closer to the origin and hence this line was adaptable to different environmental conditions; while B11377F-MR-34-2 (line no. 7) was further away from the origin which indicated that its performance was more affected by different environmental conditions. IR83835-95-1-1-3 also had high and stable yield; however, this variety was very susceptible to RTV, all races of blast and iron toxicity (Fig. 3). The

Fig. 3. Additive main effects and multiplicative interaction 2 Biplot for grain yield showing the interaction of Interaction Principal Component Axes (IPCA)2 against IPCA1 scores of 12 rice genotypes (1-12) in 9 environments (A-E; in this case some of the environments were similar since they were in the same districts).

trials were conducted under optimum conditions, where most stresses could be controlled; therefore, IR83835-95-1-1-3 had a good yield, ranged from 3.80 to 5.39 t/ha (Table 5).

Table 5. Stability parameter of nine promising lines along with check varieties across nine locations in Sumatra and Kalimantan.

No.	Genotypes/check varieties	Average yield (t/ha)	Yield range (t/ha)	Bi ²⁾	S ² di ^{y)}
1	IR84941-12-1-2	4.05	3.20-5.78	1.19	0.53
2	IR83835-95-1-1-3	4.45	3.80-5.39	0.77	0.29
3	BMIP-46-4-1	3.86	2.24-5.48	1.17	0.79
4	AGH42-2-3	3.81	2.69-4.96	0.88	0.60
5	AGH43-1-2	3.78	2.61-5.15	0.92	0.55
6	GH137-7-SKI-B	3.79	2.60-5.10	1.04	0.22
7	B11377F-MR-34-2	4.74	4.13-6.02	0.67	0.45
8	B11586F-MR-11-2-2	4.16	3.08-5.63	1.02	0.58
9	B10891B-MR-3-KN-4-1-1-MR-1	3.95	2.43-5.48	1.27	0.38
10	IR42 (check)	4.02	2.99-5.54	1.14	0.34
11	Inpara2 (check)	4.33	2.96-5.89	1.12	0.39
12	Martapura (check)	3.22	0.70-4.74	0.82	1.89
	Average	4.01		1.08	0.58

²⁾Bi: regression coefficient.

^{y)}S²di: deviation from regression.

DISCUSSION

Response to abiotic stresses

In swampy areas, iron toxicity usually occurs due to anaerobic conditions during the wet season. In acidic soils with pH below 5, aluminum toxicity will severely affect crop performance and yield. In anaerobic soils, Fe is reduced to its soluble form, Fe²⁺, and can be taken up excessively by plants. In plant tissues, Fe²⁺ participates in Fenton reactions, catalyzing the generation of hydroxyl radicals (·OH) and other reactive oxygen species (ROS) (Thongbai and Goodman 2000). The radicals cause irreversible damage to membrane lipids, proteins and nucleic acids. It could also oxidize chlorophyll and subsequently reduce leaf photosynthesis (Pereira *et al.* 2013), thereby leading to yield reductions. The typical symptoms associated with iron toxicity are leaf discoloration (bronzing) and reddish spots in the leaf blade (Tanaka *et al.* 1966). Rice grain yield losses associated with the appearance of bronzing symptoms ranged from 15% to 30%. In West Africa, rice yield losses associated with iron toxicity is estimated to be 12% to 100% (Gunawardena *et al.* 1982). Makarim *et al.* (1989) reported yield reduction as much as 90% in Batu Marta, South Sumatra, Indonesia. Based on reports from various field studies, it can be concluded that each visual bronzing

symptom score increment was associated with a yield loss of approximately 400 kg/ha (Audebert and Fofana 2009). Generally, crop damage is largest when toxicity occurs at the seedling and early vegetative stages of rice; in the worst case scenario it can lead to a complete failure of the crops (Abraham and Pandey 1989; Abu *et al.* 1989; Audebert and Sahrawat 2000). The elite lines screened in our study had scores of 1-2 of leaf bronzing and were categorized as tolerant to iron toxicity, which was similar to Mahsuri, the tolerant check, with the exception of line IR83835-95-1-1-3 (Table 1). Therefore, the eight highly tolerant lines can be cultivated in the problematic areas that have chronic iron toxicity problems, including the swampy lands.

Among the nine elite lines screened for submergence, the only line identified as tolerant was B11377F-MR-34-2 (Table 1). Interestingly, based on survey of three *SUB1* DNA markers, this line does not possess the FR13A-derived *SUB1A* tolerant allele (Fig. 1); however, *SUB1A* was highly expressed under submergence (Fig. 2). Hence, the tolerance of this line was mainly contributed by *SUB1A*. This result was similar with the finding of Septiningsih *et al.* (2012), where the *SUB1A* gene in the variety Madabaru, a moderately tolerant variety, was highly expressed under submergence, even though this variety had the ‘susceptible’ allele *SUB1A*-2. Likewise,

Singh *et al.* (2010) had reported that the susceptible *SUB1A-2* allele identified in the variety James Wee was highly expressed under submergence, despite the fact that this variety was moderately tolerant to submergence. Therefore, for a diversity study, in addition to the use of *SUB1* DNA markers it is imperative to check the expression of *SUB1A* to confirm whether *SUB1* indeed plays a role in contributing to submergence tolerance.

Response to biotic stresses

Blast is a predominant disease in rainfed areas in Indonesia, including swampy areas. The combination of well managed cultural practices, fungicides, and resistant varieties are considered to be potential remedies for rice blast disease (Wang *et al.* 2013). However, resistant varieties are considered to be the most important component and the most economically viable management strategy (Roychowdhury *et al.* 2012). During the past five years of monitoring, 26 races with various proportions of infections had been identified in Lampung, West Java, South Sumatra (swampy areas), South Kalimantan (swampy areas), and Bali. Out of the 26 races, seven races, which are races #001, 023, 033, 073, 101, 133, and 173, were identified in every year. The most infectious races were race #033, 073, 133, and 173 (Santoso and Nasution 2009). BMIP-46-4-1 is the most resistant elite line that can be planted in the worst areas of blast disease; however, this line has an average grain yield of only 3.86 t/ha (Table 2, Table 5). Potentially, this line can be used as a resistant donor to develop high-yielding varieties that are resistant to blast. On the other hand, B11377F-MR-34-2 is still a good choice since this line has the highest average yield and is not highly susceptible to blast.

To address BLB, the various pathotypes of *Xanthomonas* need to be carefully considered based on the target rice production regions and resistance genes deployed in rice varieties. Pathotype III is group of Xoo which has high virulence to Kogyoku, a differential variety for *Xa1* and *Xa12*. This group also highly infected Tetep which carries resistant genes *Xa3* and *Xa2*. But it was less virulent to Wase Aikoku harboring resistant genes *Xa3*, *Xa12*, and *Javal4*, a differential variety that carries the genes *Xa1*, *Xa2*, and *Xa12* (Suparyono *et al.* 2003). Pathotype IV is the most

virulent pathotype in Indonesia. Pathotype VIII is highly virulent to differential varieties which carry combinations of *Xa1+Xa12*, *Xa3+Xa2*, *Xa3+Xa12*, but it has less virulence to differential varieties with the pyramided genes of *Xa1+Xa2+Xa12*. These three pathotypes were widely spread in all rice production areas in Indonesia; however, Sudir (2012) and Suparyono *et al.* (2004) reported that the predominant pathotypes were different among provinces or islands. Pathotype III was common in South Sulawesi, while pathotype IV was predominant in North Sumatra. Meanwhile, pathotype VIII was the predominant group on Java Island. This means that different resistant varieties need to be deployed in each region accordingly to control the BLB disease. There are six elite lines that are better than the local checks (IR42, Inpara2, and Martapura) against BLB infection, and B11377F-MR-34-2 was the most tolerant line. Therefore, B11377F-MR-34-2 can potentially be deployed in the regions where BLB is common. The other five lines were still can be used as viable alternative as well; however, other factors such as yield and other abiotic and biotic stress components need to be taken into account.

Tungro virus is also a major disease in tidal swamp areas, especially in Kalimantan. However, thus far, no modern varieties have been identified that are resistant to tungro virus and adapted to tidal swamp areas. B11586F-MR-11-2-2 had comparable resistance with the resistant check, Tukad Petanu; while B10891B-MR-3-KN-4-1-1-MR-1 was moderately tolerant (Table 4). Therefore, these two elite lines can be deployed in the swampy areas that are prone to tungro virus infection, including regions of Kalimantan. However, other lines having high yield can still be deployed in the swampy land regions where tungro is not the predominant disease.

Adaptability of swampy rice lines

The adaptability of the lines to swampy areas was measured by analyzing the G×E interaction on grain yield. Joint regression analysis (JRA) and AMMI are the two methods that can be used to analyze G×E interactions (Asenjo *et al.* 2003). According to Eberhart and Russell (1966), stable varieties are genotypes which had high mean yield, regression coefficient (bi) close to unity and deviation from regression (S^2_{di}) near zero. A variety

having both of these two criteria as well as high yield will have a good performance in all environments. The result of our study showed that B11586F-MR-11-2-2 had wide adaptability and good average yield (4.16 t/ha); however, it is still a bit lower than that of the average of the local check, Inpara2 (4.33 t/ha). Nonetheless, B11586F-MR-11-2-2 is the only line that is highly resistant to tungro virus. Thus, it will be one of the best lines to be deployed in swampy tungro-prone areas. On the other hand, B11377F-MR-34-2 had narrower adaptability, but it had the highest yield (4.74 t/ha). B11377F-MR-34-2 was the best variety to adapt to swampy areas having acid sulphate soil, low soil fertility and complex abiotic and biotic constraints. This is based on yield data from the sulfic acid soil locations in Karang Agung. The yields on this location were 4.51 t/ha and 5.48 t/ha during WS 2012 and DS 2013, respectively. Nevertheless, based on the range of average yield in nine locations (4.13-6.02 t/ha), this variety can be deployed across many swampy land regions. Other lines which are not tolerant to iron toxicity had more unstable yields across locations.

Among the nine elite lines tested, B11377F-MR-34-2, which was derived from a double cross of Cinglonik/IRBB7//Memberamo/IR64, had the highest average yield (4.74 t/ha), which was significantly higher than those of the local check varieties (IR42, Inpara2, and Martapura). This line is also tolerant to submergence and Fe toxicity. B11586F-MR-11-2-2 is the only elite line that had high resistance to tungro virus. This line also has iron toxicity tolerance. It is hopeful that B11377F-MR-34-2 will be rapidly adopted by farmers across the swampy regions; while B11586F-MR-11-2-2 will be rapidly adopted mainly in the swampy areas which are prone to tungro virus. B11377F-MR-34-2 and B11586F-MR-11-2-2 were recently released in Indonesia as Inpara8 and Inpara9, respectively.

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