

# The Second International Conference on Genetic Resources and Biotechnology

## Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture

Bogor, Indonesia • 24–25 May 2021

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January 2022

THE SECOND INTERNATIONAL CONFERENCE ON GENETIC RESOURCES  
AND BIOTECHNOLOGY: Harnessing Technology for Conservation and Sustainable  
Use of Genetic Resources for Food and Agriculture

# Committees: The Second International Conference on Genetic Resources and Biotechnology

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## **Preface: The Second International Conference on Genetic Resources and Biotechnology**

The Second International Conference on Genetic Resources and Biotechnology, which is the continuation of the first event held in 2018, focuses on topics related to advances in biotechnology to create more opportunities for effective conservation and sustainable utilization of genetic resources for food and agriculture. This year conference's theme is Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture. The conference was organized by Indonesian Agency for Agricultural Research and Development (IAARD), Ministry of Agriculture, Indonesia, in collaboration with Indonesian Biotechnology Consortium and held on 24<sup>th</sup>-25<sup>th</sup> of May 2021 virtually due to the pandemic of COVID-19.

The conference aims to share and exchange current scientific information and technological developments on biotechnology and their applications for conservation and sustainable use of genetic, to encourage and promote quality, efficiency, and modernization of management and utilization of genetic resources, and to facilitate national and international collaboration among participants. There are five scopes discussed in this conference. They are effective management of conservation and sustainable use of genetic resources for food and agriculture, application of genomics and molecular markers for genetic resource conservation and crop adaptation to climate change, application of innovative crop improvement techniques for conservation and sustainable use of plant genetic resources for food and agriculture, plant cell and tissue culture for conservation and effective utilization of genetic resources, and the use of microbial genetic resources as biological control agents of agricultural pests and diseases, and for soil bioremediation.

Five speakers from the United States of America, Japan, India and Indonesia were invited to discuss about their expertise and knowledge on relevant subjects in the plenary sessions. This conference was attended by more than 100 participants including 75 presenters and 44 listeners worldwide. They came from diverse governmental, private, or academic institutions and also scientific communities. The presented materials have undergone peer review processes and only qualified papers were selected. Furthermore, all papers were subjected to double blind peer-review and expected to meet the scientific criteria of significance and academic excellence to be published in a conference proceedings indexed in a well-known, reputable service.

We would like to express our sincere gratitude to our speakers, presenters and all participants for their contributions in this conference. We would also like to express our appreciation for the generosity of our sponsors that support this conference: PT CropLife, PT ITS Science Indonesia, PT Fajar Mas Murni and PT Prima Instrument Analitika. Lastly, special thanks to all committee members for their exceptional work and contributions in the conference and publication.

Chair of Organizing Committee

Dr. Toto Hadiarto

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**Abstract.** Tidal swampland is now getting high priority in Indonesia agricultural development, in particular for rice production. Productivity of rice in swampland, both in tidal swamp and monotonous swamp is low compared to that in other agroecosystem due to adverse constraints, such iron toxicity, salinity, acidity, and flooding, as well as other constraints, such as socio-economic, culture, and infrastructures. Effort to increase rice productivity has been done through varietal improvement. Thirty-five swamp rice varieties have been released in the last twenty years. However, the adoption rates of those varieties are low and some varieties are rarely found in the field. Advances in biotechnology provide new tools for effective crop improvement in tidal swampland. Several rice varieties have been developed through molecular breeding approach. It is also possible to select rice lines using specific markers corresponding to *OsIRT* and *OsFer2* genes that play a role in Fe<sup>2+</sup> transport regulation and deposit in rice grain, respectively. This molecular breeding approach will not only improve rice tolerance to iron toxicity in marginal lands such as tidal swampland, but also iron biofortification of rice to mitigate iron deficiency in population that consume rice as staple food.

## INTRODUCTION

Swamplands will play important roles in future agriculture, particularly for rice (*Oryza sativa* L.) production to feed the ever-growing population in Indonesia. Swamplands are low-lying lands that are regularly flooded. It consists of two types of lands, i.e. tidal swamp and monotonous swamp. Tidal swamp lands are swamp lands that are influenced by sea tides. It can be further classified based on tidal influence into type A, B, C, and D [1]. Tidal swamplands of type A are those lands that are influenced by spring and neap tides, whereas type B are those that are influenced by neap tides only. If there are no flooding, i.e. only the rise of water table during the tides then those lands are classified as type C, while type D is not influenced by sea tides at all, and thus, basically a dry land in swampy areas. Monotonous swamplands (inland or nontidal swamp) are those land formed in the inland valley where the water comes from upstream river or rain. It has three categories based on the depth and duration of the flooding, i.e. deep, medium, and shallow monotonous swamp [1, 2].

Soil types in tidal swampland are marine sediment or fluvial and peat. Pyrites (FeS<sub>2</sub>) naturally occurring as a result of sulphate reduction by bacteria. In waterlogged or anaerobic conditions, the pyrite is stable. However, if it is exposed to aerobic condition, it will be oxidized and deactivated causing the soil to be very acidic and release Fe<sup>3+</sup>, which may cause excessive soluble iron in flooding condition. The availability of iron is dependent on the soil pH. Iron becomes less soluble in higher pH and it can be found in the form of insoluble ferric oxides. In contrast, iron becomes more soluble in low pH and they can be readily absorbed by the plant roots. The iron becomes toxic to plant, particularly rice and may cause bronzing in leaves resulted from the oxidation of polyphenol. In addition, excessive iron may also cause stunted roots in plant, delaying harvest, and decreasing biomass production. Reduction of rice yield caused by iron toxicity may vary from 12–100% [3]. Although iron is toxic in excessive amount, it is an essential element in human diet, as its deficiency may lead to anemia and other disorder diseases.

The rice grain Fe enrichment or biofortification, therefore, is very important in reducing iron deficiency in human population. It was estimated that up to 30–50% of world population affected by Fe deficiency [4]. Fe has several vital functions in human metabolism, such as synthesis of hemoglobin and formation Fe-containing enzymes, which are important for energy production, immune defense, and thyroid function [5, 6]. Since rice is a staple food for a half of world population, mostly in poor countries. Fe biofortification will significantly reduce Fe and possibly other nutrient deficiencies.

In this paper, to get a better perspective on tidal swamp rice, we reviewed the availability of traditional and improved tidal swamp varieties in Indonesia and the progress in molecular breeding for iron toxicity tolerance and biofortification of tidal swamp rice.

## CURRENT STATUS OF AVAILABLE TIDAL SWAMP RICE VARIETIES

### Traditional Varieties

Traditional rice cultivation in swampland can be represented by the tradition in South and Central Kalimantan [7]. Traditional tidal swamp rice cultivars have become an indispensable part of the local culture and traditions of the people in this region [8]. Those cultivars are mostly photoperiod sensitive, i.e. they can only be flowering when the day length shorter than the critical day length. The traditional cultivation practices in tidal swampland are farmers adaptation to photoperiod sensitivity of the traditional varieties. Basically, there are three steps in seedling and planting of rice in those practices, i.e. *taradak*, *ampak*, and *lacak*. *Taradak* is a process of planting seeds in upper or drier land mostly in home yard areas of farmers. After 21 days grown at the *taradak*, the seedlings are then transplanted to the wetland with non-spacious planting distant, which is called the *ampak* step. The *lacak* step is the transplanting of the seedling from the *ampak* step to the tidal swamp rice field. This *lacak* step is taken when the height of the seedling is suitable for being planted on the swamplands at particular water height.

In addition to their photoperiod sensitivity, traditional tidal swamp rice varieties have several characteristics, i.e. tolerant to iron toxicity, salinity or waterlogging, depending on type of tidal swamp it has been developed and cultivated for generations. In South Kalimantan Province, Indonesia, these cultivars have been observed morphologically and showed a close relationship with the local culture. A total of forty traditional rice cultivars with different morphological characteristics are still preserved sustainably by the local farmers in this region along with their local culture and traditions [8]. Some traditional foods made by the local people use some of the local rice as basic ingredients. Other characteristic of traditional tidal swamp rice that determines its preference by local people in South and Central Kalimantan is the grain quality. Most traditional tidal swamp rice has long and medium size grain in addition to photoperiod sensitivity, tolerance to abiotic and biotic stress in tidal swamp environment. Such grain quality will have dry-cooked rice which are preferred by local consumers, and fit the traditional food as well as main dish of local people.

Diversity of traditional tidal swamp rice cultivars in South and Central Kalimantan has been studied phenotypically and molecularly [8–10]. One study revealed that traditional tidal swamp rice cultivars showed a relatively low phenotypic diversity, both the qualitative and quantitative traits. However, some traits exhibit high diversity, such as the secondary branches of panicle, awn distribution, the grain ratio, number of tillers, and plant height. The higher the number of tillers, the higher the yield can produce. Traditional varieties, *Lakatan Pacar* and *Siam Babirik*, were the cultivars with the highest plant architecture. Crop productivity is determined by several major traits, i.e. number and weight of panicles per plant unit, number of panicles, panicle length, number of days of flowering, plant height, number of grains per panicle, number of fertile grains per panicle, and grain weight [11]. *Ganal Perak* is a tidal swamp rice cultivar that shows higher grain weight than others.

### Improved Varieties

Improvement of tidal swamp rice are mostly done through crossing of traditional cultivar that are tolerant to abiotic stress and high yielding varieties introduced from other regions. As of 2018, there are 35 improved varieties of swamp rice that have been released, consisting of 21 tidal swamp rice, 4 monotonous swamp rice, and 10 varieties that are adapted to both tidal swamp and monotonous swamp, as well as irrigated rice field [12–14]. The yield potency of the improved varieties increased gradually by the time of its release; indicating the improvement in breeding program. This improvement, however, did not necessarily in line with the increase in rice productivity at four provinces which are dominated by tidal swamp as illustrated at the graph in Fig. 1 below.

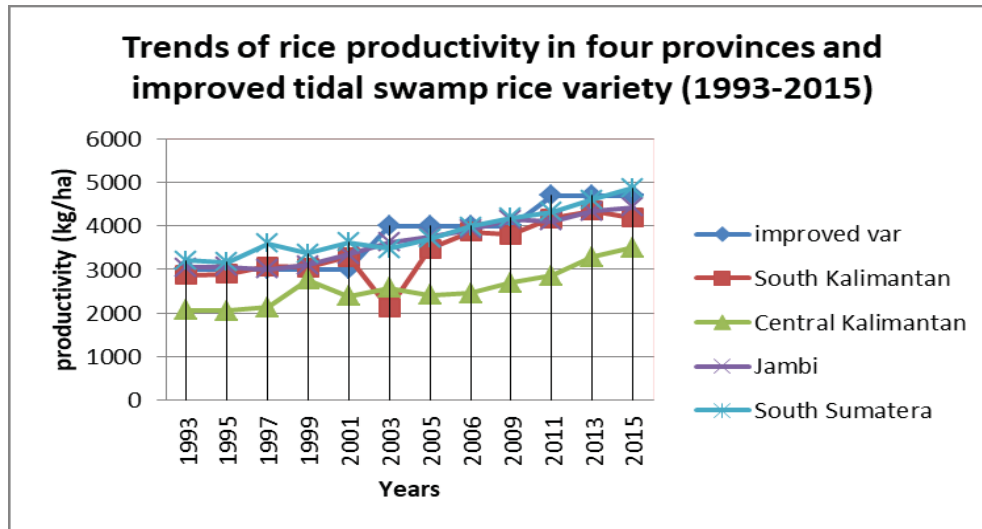


FIGURE 1. Productivity growth of new improved rice variety in tidal swamp areas at four provinces in Indonesia.

Assuming that an improved variety needs at least 2 years before its adoption by farmers and contributing to the increase of productivity, we compared the productivity in four provinces at certain year with potential productivity of a new variety released 2 years before the indicated years and put the trend from 1993 to 2015 in Fig. 1 above. The graph shows that in almost all years, the productivity of rice in tidal swamp-dominated provinces is below the productivity as it should have been if the new variety was adopted. This indicated low adoption rate of improved variety of tidal swamp rice. A study revealed that adoption rate of improved varieties in tidal swampland ranges between 20–40%, but as many programs include the use of improved varieties seeds in aid packages, now the adoption of improved varieties has reached more than 60% of the planted area [15]. Several aspects have been identified as constraint to adoption of improved varieties, e.g. most improved varieties are planted twice a year which are not compatible with farmers tradition that used to harvest rice once a year with limited maintenance and more preferable rice grain by local consumers in South and Central Kalimantan [16].

## IMPROVEMENT ON TOLERANCE TO IRON TOXICITY AND IRON BIOFORTIFICATION OF RICE

Iron toxicity may constrain plant performance [17]. Iron toxicity is one of the major constraint to rice cultivation in tidal swampland. Several traditional varieties and breeding lines have been tested for iron toxicity [18–20] and several Fe-tolerant varieties have also been developed by conventional breeding in many countries [21]. These varieties, like other improved varieties for tidal swamp rice, have not yet been fully adopted by farmers. It is therefore important to continuously improve rice tolerance to iron toxicity coupled with improvement on other important characters.

Fe toxicity tolerance mechanisms and its associated markers have been identified [6, 22–24]. Those tolerance mechanisms consist of Fe exclusion from the roots, Fe retention in roots and decrease of Fe translocation from roots to shoot, Fe compartmentalization in shoots and enhance Fe storage and detoxification of reactive oxygen that may cause damage to leaves. Fe exclusion inhibit root Fe absorption by preventing the expression of the Fe uptakes genes. In more severe excess of Fe, its retention in roots and prevention of translocation to shoots are a defense mechanism of plants. Fe compartmentalization is the sequestration of the Fe excess in the safe form in shoots. If the excess of Fe cannot be completely sequestered, Fenton reactions are catalyzed to generate reactive oxygen that may cause damage to leaves and consequently reduce yield. In this condition, the reactive oxygen will be detoxified to avoid the damage to plant cells.

Different genotypes respond differently to excessive iron and ability to translocate it into rice grain [25]. Several genes have been identified for its role in regulating the Fe exclusion, retention, translocation, and storage in rice grains [22, 26–30]. In the excess of soluble Fe<sup>2+</sup>, like in tidal swampland, rice uptakes Fe<sup>2+</sup> iron directly from the soil, in addition to uptaking Fe<sup>3+</sup> in chelated form that involves several chelating molecules, like other graminaceous

plants, and transport it into roots. This processes regulated by iron transporter (IRT) genes, *OsIRT1* and *OsIRT2* [31]. The excessive amount of absorbed Fe in roots will be transported to leaves by xylem via transpiration stream and will lead to cellular Fe overload in plant tissue. Once in plant tissues, the Fe<sup>2+</sup> ions are stored in cellular compartment, such as ferritin and vacuoles. Ferritin is a plastid localized complex protein that can accommodate up to 4,500 Fe atoms and stores Fe in nonreactive manner [32]. The rice Ferritin genes, *OsFER1* and *OsFER2*, are strongly upregulated in roots and shoots in excessive iron [33], so that the iron can be stored in safe and bioavailable form. Vacuolar membrane transporters encoded by *OsVIT1* and *OsVIT2* genes involves in the storage of iron in vacuoles of flag leaves and its inhibition resulted in increase of iron in seed [34].

Rice can be an ideal species for Fe biofortification since it is a staple food that is especially important for developing countries, grown in flooded soils where Fe availability is high [35], and its mechanisms of Fe absorption, translocation, and homeostasis are better understood than other species [27, 36, 37]. Iron biofortification of rice can be done through agronomic practices, conventional breeding, and genetic engineering [38–40]. Agronomic biofortification or ferti-fortification, involves iron uptake and translocation into edible part of plants. Application fertilizer either on soil or foliar application is common agronomic practice to enhance iron uptake. Conventional breeding, although has resulted in several high iron content varieties, requires intensive crossing and selection, which may take times. There are several approach in genetic engineering for developing biofortified varieties, i.e. (1) improving iron storage via ferritin genes [41], (2) enhancing iron transport via *NAS* gene [42], (3) enhancing iron influx via *OsYSL2* gene [43, 44], (4) enhancing iron uptake and translocation via *IDS3* gene [45], and (5) enhancing iron translocation via silencing *OsVITs* gene [34]. Several transgenic rice varieties with significant increase in iron content in rice grain have been developed by those approaches [27, 46].

Specific molecular markers for assisting the selection of breeding lines corresponding to *OsIRT-2* and *OsFer2* genes that control Fe<sup>2+</sup> transport regulation and its storage in rice grain [47, 48] have been identified. Genotype profiling of seven promising tidal swamp lines tolerant to Fe toxicity, confirmed that they are polymorphic to sensitive line detected using *OsIRT2* marker and also positive for *OsFer2* allele gene detected on their profile. Small size allele (200 bp or less) co-segregated with tolerance characters while large size allele (300 bp or more) co-segregated with sensitive characters [49]. Several promising lines of iron toxicity tolerance rice with considerable high iron bioavailability have been identified and presented in Table 1.

**TABLE 1.** Several Fe-biofortified promising rice lines have been developed [49].

<b>Lines</b>	<b>Yield (t/ha)</b>	<b>Yield average (t/ha)</b>	<b>Harvest (DAP)</b>	<b>Superior characters</b>
BioFe-1R	6.15	4.00	103	Tolerant to Fe toxicity, sticky, total Fe content on rice (50.4 ppm), bioaccessibility (33%)
BioFe-5R	5.80	4.80	101	Tolerant to Fe toxicity, fluffy, total Fe content on rice (31.0 ppm), bioaccessibility (40%)
BioFe-10R	7.00	4.80	104	Tolerant to Fe toxicity, light, total Fe content on rice (51.7 ppm), bioaccessibility (34%)
BioFe-11/14R	6.30	4.40	105	Tolerant to Fe toxicity, light, total Fe content on rice (32.2) ppm, bioaccessibility (50%)
BioFe-47F	7.60	4.70	101	Tolerant to Fe toxicity, highly sticky, total Fe content on rice (40.2 ppm), bioaccessibility (33%)

DAP = days after planting.

## CONCLUSION

Tidal swamp rice crop improvement has been done for several years and resulted in 35 improved varieties. The adoption rate of these varieties is relatively low as indicated by the increase of rice productivity in tidal swampland-dominated provinces in Indonesia, such as South and Central Kalimantan, that are not in line with the increase in productivity of the improved varieties. Farmers and consumers in South and Central Kalimantan prefer to cultivate and consume local varieties because of their suitability with local culture and tolerance to adverse abiotic stress such as iron toxicity. Effort to increase tolerance to iron toxicity and productivity is continuously done by conventional breeding. Advances in biotechnology made possible to identify genes that control the absorption and translocation of iron in plant, such genes then can be used as markers to select preferable rice lines. Such markers-assisted selection will identify the lines tolerance to iron toxicity and have high iron content in their grains. Fe biofortification of rice might mitigate the Fe deficiency in human population.

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