Sustainability of Populations of Wild Rice Species in Natural *in situ* Conservation Sites of South and West Sulawesi

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Abstract: *Exploration and identification of wild rice species (Oryza spp.) was carried out in South and West Sulawesi area of the Sulawesi Island in Indonesia. We collected several accessions of wild rice in these surveys and identified species of accessions through DNA analysis. Two accessions of wild rice were collected in Palopo (IS-03) and Malangke (IS-20) of South Sulawesi and one accession was collected in Mamuju of West Sulawesi (IS-23). Total DNA was isolated from leaves of each accession using a modified CTAB method. Then, DNA fragment containing intron 19 sequence of PolA1 gene was amplified by PCR using a pairs of primers, 19ex5P and 20ex3P. DNA sequence of the amplified PCR product was determined by direct sequencing method. The result showed that these three wild rice accessions collected in South and West Sulawesi were identified as Oryza officinalis. Sequence analysis also showed that there were two types of the intron 19 sequences in O. officinalis. Two accessions from Palopo and Mamuju had the intron 19 sequence (298 bp) that was identical to that of O. officinalis accession (W0614) from Prome in Burma. In contrast, the accession from Malangke had the sequence (283 bp) that was shared with W0002 accession from Bangkok in Thailand. The habitats of O. officinalis were swamp or a temporary flooded plain, for example at the edge of the ditch and the rivers that are flooded in the rainy season. Sustainability of populations of this wild rice species in natural in situ conservation sites is now dangerously threatened by suburbia town expansions during the last five years.*

Keywords: *Wild Rice Species, Oryza, PolA1, RNA polymerase I largest subunit*

1. Introduction

Two cultivated rice species (*Oryza sativa* L. and *O. glaberrima* Steud.) have 22 species of wild relatives (Khush 1997). Indonesia is considered as a center of area where the genus *Oryza* had been originated (Vaugham *et al.*, 2003). Seven *Oryza* wild species are spread over the larger islands in Indonesia, such as Sumatra, Kalimantan and Java. Wild rice is an important resource of genetic diversity for the advancement of rice breeding in the future. Various agronomic important characteristics, such as resistance to pests and viruses, of wild rice species have been introduced into cultivated rice species (Li *et al.*, 1990).

The existence of wild rice species (*Oryza* spp.) has been reported in Indonesia since 1800. The first wild rice species found in Indonesia was *O. meyeriana*, which inhabited in Java Island. *Oryza meyeriana* was discovered by Zoliger in 1842 (Vaughan, 1994 in Hanarida, 2001). According to Vaughan (1994) in Hanarida (2001), seven *Oryza* species, *O. meyeriana, O. granulata, O. rufipogon, O. longiglumis, O. officinalis, O. ridleyi,* and *O. schlecteri,* habitat and distributed through almost all major islands in Indonesia.

Sulawesi Island is an unique area because Asian-type *Oryza* species, such as *O. rufipogon* (A genome), *O. officinalis* (C genome), and *O. granulate* (G genome), coexist with Pacific-type *Oryza* species, such as *O. meyeriana* (G genome), *O. longiglumis* (HHJJ), *O. ridleyi* (HHJJ), and *O. schlecteri.*

Most recent data on the existence of wild rice species in the Sulawesi Island was reported by Vaughan 1994 in Hanarida 2001), whereas there has been considerable change in the environment as the impact of development in the past 15 to 20 years. Application of the green revolution and the opening of a massive land for the benefit of intensive agriculture, plantation estates, residential and industrial, have damaged the environment and natural ecosystems, hence it is most likely to cause the loss of wild rice species as well as other plant species.

Based on the above description, we carried out the exploration and identification of accessions in order to try mapping the latest distribution of wild rice species (*Oryza* spp.) in South and West Sulawesi provinces.

2. Material and Method

The exploration of wild rice species was carried out in area where their distributions were previously reported in South and West Sulawesi Provinces. The research was conducted from in 2009 until 2010 and 2014.

Each exploration was conducted by driving a car and hitch hiking through provincial roads and then followed district roads to explore semi-shaded places, which were preferred habitat of wild rice species. Based on information from the study of literature, we learned that the habitats should be on the outskirts of the river, near the water springs and marshes land. Information on sightings or knowledge of this species is also obtained from farmers and residents we met by showing pictures of various species of wild rice.

Species-identification of the collected accessions was performed by DNA analysis. This is to avoid the mistakes of identification because of the huge morphological similarity between some species of wild rice. The total DNA was isolated from the leaves of each accession using a modified CTAB method. DNA fragment containing intron 19 sequence of *PolA1* gene was amplified by Polymerase Chain Reaction (PCR) using a pair of primers, 19ex5P and 20ex3P (Figure 1). PCR was performed using *ExTaq* DNA polymerase (TaKaRa Co., Shiga, Japan) according to the manufacturer's instructions. The thermocycling profile consisted of an initial denaturation step at 94ºC for 3 min, followed by 35 cycles of denaturation at 94ºC for 1 min., annealing at 59ºC for 1 min and extension at 72ºC of 2 min in a PTC200 thermocycler (MJ Research Inc., Waltham, MA, USA).

The amplified PCR products were subjected to 3% (w/v) agarose gel electrophoresis and purified using the QIA quick PCR purification kit (Qiagen Inc., Valencia, CA, USA). The purified PCR product was then sequenced directly using the same primers used for PCR amplification in an automated DNA sequencer (ABI310; Life Technologies) with a BigDye Terminator Cycle Sequencing Kit (Life Technologies).

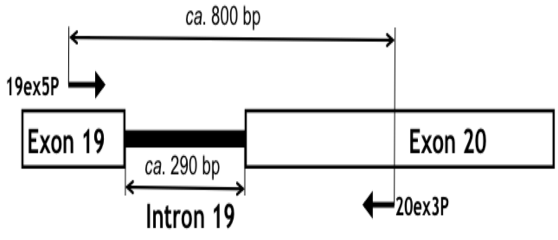


Figure 1. DNA fragment containing intron 19 sequence was amplified by PCR using a pair of primers, 19ex5P: 5'-CGCTGGACGGGGTGAGATGAATG-3 'and 20ex3P: 5'-CATAATCCATCTCATCTGTTTCTTG-3'

3. Results and Discussion

Two accessions of wild rice were collected in Palopo (IS-03) and Malangke (IS-20) of South Sulawesi and one accession was found in Mamuju of West Sulawesi (IS-23) (Figure 2). The three accessions were identified as *O. officinalis.* Although returned visit during 2014 still confirmed the existence of the species, there were decreased number compared than the original population in Malangke. Whereas in Mamuju, wild rice species has disappeared completely due to city development because Mamuju is the capital city of new province of West Sulawesi, which was split from South Sulawesi in 2004. Hence, development of offices and housing estate, and construction of roads destroyed habitats where wild rice species used to be present.

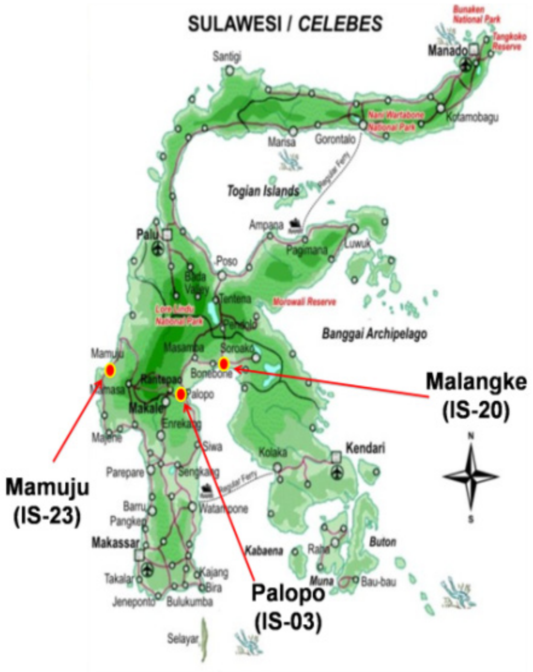


Figure 2. Locations of wild rice accessions collected in Sulawesi

The wild rice species, *Oryza officinalis* (C genome), was found in swamp habitats, as well as on the plains were flooded temporarily, e.g. on the edge of the ditch and the river that were flooded in the rainy season (Table 1). In contrast to cultivated rice species, *O. sativa* (A genome), which can generally grow in full sunshine places, *O. officinalis* is collected in semi-shaded places.

Tabel 1. Description for habitats of *Oryza officinalis* accessions collected in West and South Sulawesi, Indonesia

Name of Suburbia	Village	Location Descriptions			
		Coordinate	Altitude (m)	Habitat	Descriptions
Mamuju Regency, West Sulawesi Province (IS-23)					
Kalukku	Beru-beru (BB)	S 02°31’36,2’’ E 119°04’50,2’’	15	Swamp, and the edge of the trench	Growing up on the edge of a small swamp, the water levels up to 12 cm, and the edge of a dry moat, but in the rainy season flooded.
	Tasiu (TS)	S 02°32’53,6’’ E 119°04’25,2’’	15	Swamp	Grow at the edge of the swamp the water levels until 20 cm, marsh flooded during the year
	Sinyonyoi (SY)	S 02°33’60,4’’ E 119°04’10,3’’	15	Swamp	Grow in swamp habitat sago tree height to 15 cm of water. Marsh flooded throughout the year
Luwu Regency, South Sulawesi Province (IS-03) Palopo?					
Kamaenre	Saluparemang (SP)	S 03°17’50,5’’ E 120°22’41,7’’	10	Gardens of palm and banana, around the stagnant water, and dry moat	Grows around the stagnant water in the rainy season, dry moat, but in the rainy season the water flowed
North Luwu Regency, South Sulawesi Province (IS-20)					
Malangke	Pattimang (PG)	S 02°31’36,2’’ E 119°04’50,2’’	15	Flooded, cocoa garden suburb, a dry moat	Grows at the edge of the garden cocoa, on a dry moat, in some parts still seem stagnant water

Sources: Primary data, 2010

According to Morishima and Oka (1997), *O. officinalis* can grow in an environment with high rainfall almost throughout the year, and is widespread in equatorial regions where there is no clear boundary between the rainy season and dry season. Survey conducted by Sato *et al*. in Cambodia (1999) and Myanmar (1998-2000) also found this species generally grow in wet habitats, ditch, swamp, around small springs, along the edge of a lake or river.

In many locations, *O. officinalis* is growing along with the plants of the family Leguminosae, among others *Gliricidia sepium*, *Leucaena leucocephala*, *Calopogonium mucunoides*, and *Mimosa invisa* which could fix nitrogen from air. Research conducted by Sato (2007) also showed that *O. officinalis* was associated with endophytic bacteria that help the fixation of nitrogen supplied N to the rice plants.

Although morphological observations of three accessions, collected in three places (arrow) in the Sulawesi Island, suggested as *O. officinalis*, it was difficult to distinguish between diploid CC genome species *O. officinalis* and tetraploid BBCC genome species *O. minuta*.

To confirm the species of the three accessions, analysis of intron 19 sequences of *PolA1* gene was carried out because its sequence showed species-specific variation in the genus *Oryza* (Takahashi *et al.,* 2009) and *Triticum-Aegilops* (Rai *et al.,* 2012).

The *PolA1* gene is single-copy per haploid genome and encodes the largest subunit of RNA polymerase I, which plays an important role in the synthesis of 45S ribosomal RNA.

Takahashi *et al.* (2009) reported that the length of *PolA1* intron 19 sequence was differentiated among genome-types in the genus *Oryza*. The length of the intron 19 sequence was 290 bp in AA and CC genomes, 220 bp in EE and GG genome, 350 bp in FF genome, and 500 bp in BB genome (Figure 3). Three accessions were collected in Sulawesi (South and West Sulawesi) have the same intron length with CC genome species, *O. officinalis*, and clearly differentiated from BBCC genome species, *O. minuta*.

Sequence analysis showed that there were two types of the intron 19 sequences in *O. officinalis* (Figure 4). Two accessions of Palopo and Mamuju have a sequence (298 bp) that was shared with W0614 accession of *O. officinalis* from Prome in Burma. Contrary, one accession of Malangke and W0002 accession from Bangkok in Thailand shared another sequence (283 bp) of *O. officinalis*. This result suggests that these two types of *O. officinalis* have a wide range of distribution from central Asia to Pacific islands.

Figure 3. Agarose gel pattern of PCR products containing *PolA1* intron 19 sequences in *Oryza* species. PCR products showed length variations which were specific to genome-types in the genus *Oryza* (a) and three accession collected in this exploration showed the same sized fragments specific to CC genome species (b).

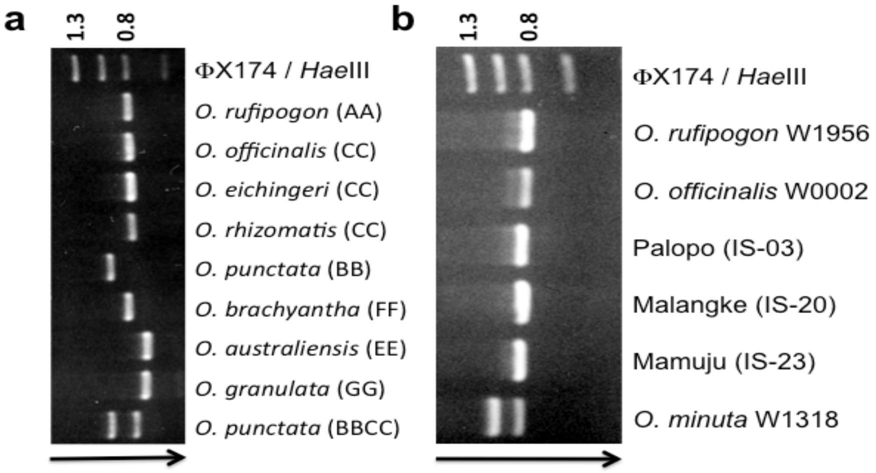
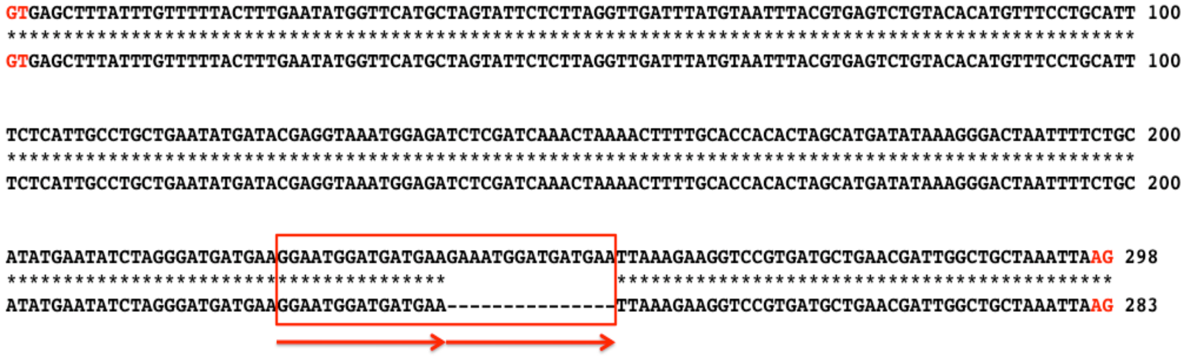


Figure 4. Two types of *PolA1* intron 19 sequences in *O. officinalis* accessions found in the Sulawesi Island. Insetion/deletion (indel) of imperfect tandem repeat (15 bp) was found between upper (298 bp: Palopo and Mamuju, and W0614, Burma) and lower (283 bp: Malangke, and W0002, Thailand). DNA sequences of both types were identical except an indel of 15 bp tandem repeat.



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4. Conclusion

The wild rice species collected in South and West Sulawesi was identified as *O. officinalis*. *In situ* conservation from first sighting in 2009/2010 was re-affirmed in dry season of 2014. The habitats of wild rice found were in swamps, or on a temporary basis flooded plains, for example at the edge of the ditch and the river that flooded in the rainy season. However, the habitats would soon be destroyed since the area has been under development for the town's expansion plan by suburbia government. Therefore, more comprehensive development is emphasized.

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