ORYZA LONGISTAMINATA'S CHROMOSOME SEGMENTS ARE RESPONSIBLE FOR AGRONOMICALLY IMPORTANT TRAITS FOR ENVIRONMENTALLY SMART RICE

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Abstract

Modern agriculture using excess fossil energy has led to high productivity of crops. However, it has induced several adverse effects on the global environments. For sustainable global environments in the 21st century, environment-smart agriculture systems (ESAS) should immediately be developed. One possible idea for ESAS is to breed low-input-adaptable (LIA) crops. In low-input conditions, plant biomass decreases significantly, resulting in lower yields. *Oryza longistaminata*, a wild species grown only in Africa, possesses several latent useful traits including a vigorous biomass observed in low input conditions. This is an important trait for LIA crops. Hence, this study aims to analyze QTLs for several important traits for large biomass of *O.longistaminata*. MwM, *O. longistaminata*, collected at a valley near Mombasa Kenya, was successfully crossed with T-65, a japonica variety. LIA candidates were selected from selfed pedigree of the cross under non-fertilized conditions of IPSR. After their characterisation, these lines were found to possess important traits for high productivity in low-input conditions. Genotyping using genome-wide SSR markers demonstrated that LIA-1 carries several chromosome segments of MwM. QTL analysis for several important traits for large biomass of MwM was conducted in F2 of the cross between LIA-1 and Norin 18. The results suggested that MwM-derived chromosome segments locate QTLs for important traits for LIA rice. This information is useful to breed New Basmati which could achieve higher productivity in low input conditions in Kenya.

Key words: LIA, *Oryza longistaminata*, QTLs, chromosome segment

1   Introduction

Rice is an important cereal crop worldwide. It accounts for over 21% of the calorific needs of the population of the world (Fitzgerald *et al.*, 2009). It is needed for achievement of higher yields of cereal crops. At the green revolution, semi-dwarf crop varieties were developed and led to achievement of higher productivity. However, these varieties require high input conditions for their optimum production. The high input condition in modern agriculture in return has led to negative impacts to the environment; soil acidification, pollution of rivers, lakes and ground water and emission of greenhouse gases that influence global warming (Ravishankara *et al.*, 2009). Recently, the price of inputs especially fertilizers has been on an upward tread mainly due to limitation in fossil fuel (http://news.mongabay.com/2008/0220-fertilizers.html). As a result, the cost of production of most crops has gone high leading to high crop prices. Then, farmers have been using inadequate amounts of inputs, resulting in food shortages. In the 21st century, low-input agriculture systems using crop varieties adapted to low-input conditions is important for ideal sustainable agriculture. Therefore, environment-smart agriculture systems (ESAS) should immediately be developed. This can be achieved by maximizing the crop-ability responsible for tolerance to various abiotic and biotic stresses. One way of achieving this is through breeding of Low Input Adaptable (LIA) crops. The utilization of genetic resources and distantly related wild relatives is effective for this. Wild species are tremendous gene reservoirs for domesticated rice improvement, as they possess many desirable traits (Xiao *et al.*, 1998). In low input conditions, the biomass of crops is significantly reduced and resulting in low yields. *Oryza longistaminata* is an AA genome wild species of rice that is distantly related to *Oryza sativa.* It is broadly distribute throughout tropical Africa. It is a perennial species characterized with long anthers, self-incompatibility, allogamy and strong rhizome. In low input conditions, it shows a vigorous biomass (Sacks *et al.*, 2003). This large biomass of *O. longistaminata* is an important trait for breeding low-input adaptable rice. Further, it has been shown that it is tolerant to bacterial leaf blight (Kush *et al.*, 1990; Sacks *et al.*, 2003). However, its utilization in breeding programs for rice improvement has been very minimal due to developed crossing barriers (Chu and Oka, 1970). Nevertheless, *O. longistaminata* was successfully crossed with T-65, a japonica variety, and large biomass lines were selected under non-fertilized conditions at Institute of Plant Science and Resources (IPSR), Okayama University, Japan. These lines are expected to possess high productivity in low-input conditions. In this study, the selected LIA lines were characterized for important agronomic traits and crossed with Norin 18. The F2 of the cross between LIA and Norin 18 was analyzed for important QTLs that are responsible for the high productivity in low input conditions of LIA.

2   Materials and Methods
2.1   Plant Material

LIA lines were bred from the progeny of the cross between MwM, *O. longistaminata* collected at a valley 10km north from Mombasa, Kenya and Taichung 65 (T-65), a japonica variety. Six LIA lines (LIA-1, LIA-2, LIA-3, LIA-4, LIA-5 and LIA-6) were selected at F11 generation. A population of 197 F2 plants of the cross between LIA-1 and Norin 18 were used for QTL analysis. Selected LIA lines and F2 plants together with Norin 18, a japonica low input adaptable rice variety, were grown in a non-fertilized paddy field kept under non-fertilized conditions for over 20 years at IPSR, Okayama University, Japan. LIA lines and Norin 18 were also grown under fertilized conditions with 5kg of N.P.K. respectively, as a control. Heading date and various agronomic traits at mature stage were measured: Culm length, panicle length, number of panicles per plant, mean panicle weight, number of primary branches, number of secondary branches, number of spikelets per panicle, spikelet fertility and weight of 100 grains.

2.2   QTL Analysis

Genotyping of MwM and LIA-1 were performed using 111 SSR markers selected genome-widely along with T-65, No.18 and F1 of the cross between LIA-1 and No.18. Genotyping of each F2 plant was done

using 35 SSR markers genome-widely selected. A genetic linkage map of 35 SSR markers was constructed using MAPMAKER. Composite Interval mapping (CIM) was performed for QTL analysis in F2 plants using the software Windows QTL Cartographer 2.5. In all the investigated traits, the LOD threshold was determined at the experiment-wise significance level of 0.05 by computing 1000 permutations.

3   Results

LIA candidates selected for large biomass under non-fertilized conditions were characterized by long culm, few tillers, large panicle, a large number of spikelets and large grain size (Table.1). However, spikelet fertilities were not high. Several agronomic traits of LIA-1 were compared to those of No.18 under fertilized and non-fertilized conditions (Table 2). Some characters of LIA-1 under non-fertilized conditions were larger than those under fertilized conditions. These characteristics are important for high productivity under non-fertilized conditions; hence the name Low Input Adaptable (LIA) rice is used. Polymorphisms of genome-wide SSR markers showed introgressed chromosome segments of MwM into LIA-1 in chromosomes, 1, 2, 3, 6, 8, 10 and 11 (Fig.1). In particular, most of the short arm of chromosome 6 of MwM is introduced into LIA-1.

All the traits measured showed normal distribution except for spikelet fertility. Significantly positive correlation was observed between stem diameter and panicle related traits, between number of spikelets and secondary branches, and between spikelet fertility and panicle weight (Tab.3). A total of 32 QTLs were mapped. These QTLs are distributed on chromosome 3, 5, 6, 8, 9, 10 and 11 of LIA-1. Important, many QTLs (CL, PL, PW, SB, NSP, SF, GW, WG, and HD) are located in chromosome six. In some chromosome locations more than one QTL was mapped on the same location (Fig.2). Thus, yield related QTLs were found to be located in MwM introgressed segment regions of LIA-1.

4   Discussion

Previously, a lot of research work involving wild relatives of rice was focused on exploring disease resistance. Major genes for resistance to grassy stunt virus and those to bacterial leaf blight have been successfully transferred from wild rice species; *Oryza nivara* and *Oryza longistaminata*, respectively, to cultivated rice (Kush *et al.*, 1977, 1990). To overcome the yield plateaus currently being experienced in rice breeding due to a narrow genetic basis of parental materials, many breeders are exploiting and utilizing the favorable alleles of the wild rice e.g *Oryza rufipogon, Oryza nivara* and *Oryza glumaepatula* (Xiao *et al.*, 1998; Brondani *et al.*, 2002). In this study, the large biomass of *O. longistaminata* was focused on the utilization as genetic resources for achievement of high productivity under low input conditions. As a result, *Oryza longistaminata* segments were found introgressed into LIA-1. In particular, the short arm of chromosome 6 of MwM is introduced into LIA-1. Relatively, few segments of MwM were introgressed into LIA-1 chromosomes. However, LIA-1 was shown to exhibit the large biomass characteristics in low input conditions as in MwM. This suggests that the large biomass characteristics may be controlled by small sections of the MwM genome. These characteristics are important for high productivity under non-fertilized conditions. In most of the parameters measured there were no significant change between the LIA-1 population grown in non-fertilized conditions and that grown in fertilized conditions. A significant decrease was only observed in culm length and panicle length of the plants grown in non-fertilized conditions as compared to those grown in fertilized conditions. However, a significant increase was observed in culm base diameter. Interestingly, culm base diameter, flag leaf length, mean panicle weight, number of primary branches, number of secondary branches and number of spikelets showed a higher mean in the population that was grown in non-fertilized conditions as compared to that grown in fertilized conditions. Tian *et al.*, (2006) also found that QTLs derived from *O. rufipogon* introgressed segments in a set of 159 introgressed lines (ILs) were usually associated with an improvement of the target trait. Traits whose QTLs were identified in this study included: Heading date

(HD), Culm length (CL) Panicle length (PL), No. of Panicles (P), Stem diameter (SD), Primary branches (PB), Secondary branches (SB), No. of spikelets per panicle (NSP), Spikelet fertility (SF), Weight of 100 grains (WG), Length of grain (LG), and Grain width (GW) (Fig 2). A majority of these QTLs mapped were found located on the introgressed sections of *O. longistaminata* in LIA-1 chromosomes. To explore the genetic resources from wild rice, several populations derived from combinations between diverse cultivars and wild rice have been used for QTL mapping. Numerous traits have been investigated and many QTLs identified (Moncada *et al.*, 2001; Li *et al.*, 2006; Yoon *et al.*, 2006). In particular, Xiao *et al.* (1998) detected a total of 68 QTLs for 12 traits using a back cross population derived from a wild rice (*Oryza rufipogon*) and cultivated rice. This study has shown that *O. longistaminata* contains important QTLs for yield and yield-related traits. These QTLs are important and can be utilized to breed for high yielding rice varieties under low-input conditions.

5   Conclusion

Oryza longistaminata is difficult to cross with *Oryza sativa* due to the presence of crossing barriers. However, it has high potential for improvement of rice. This study has shown that recombinant inbred lines successfully developed from *Oryza longistaminata* and *Oryza sativa* carry important QTLs that can be utilized in breeding programs. These RILs can be used to breed new rice varieties that are high yielding in low input conditions.

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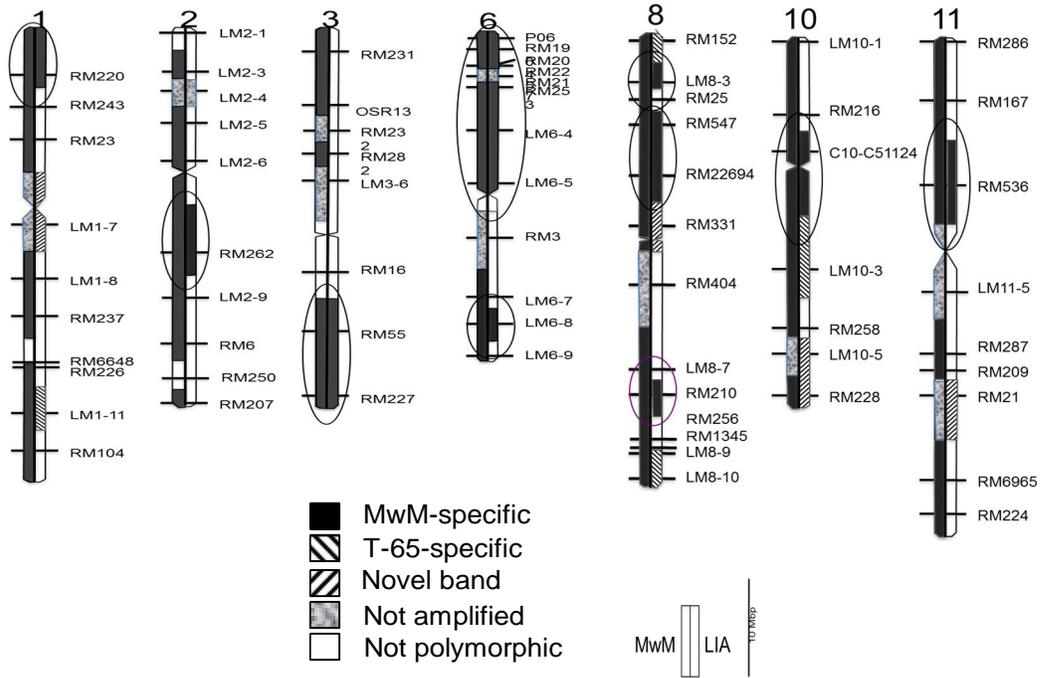
Appendices

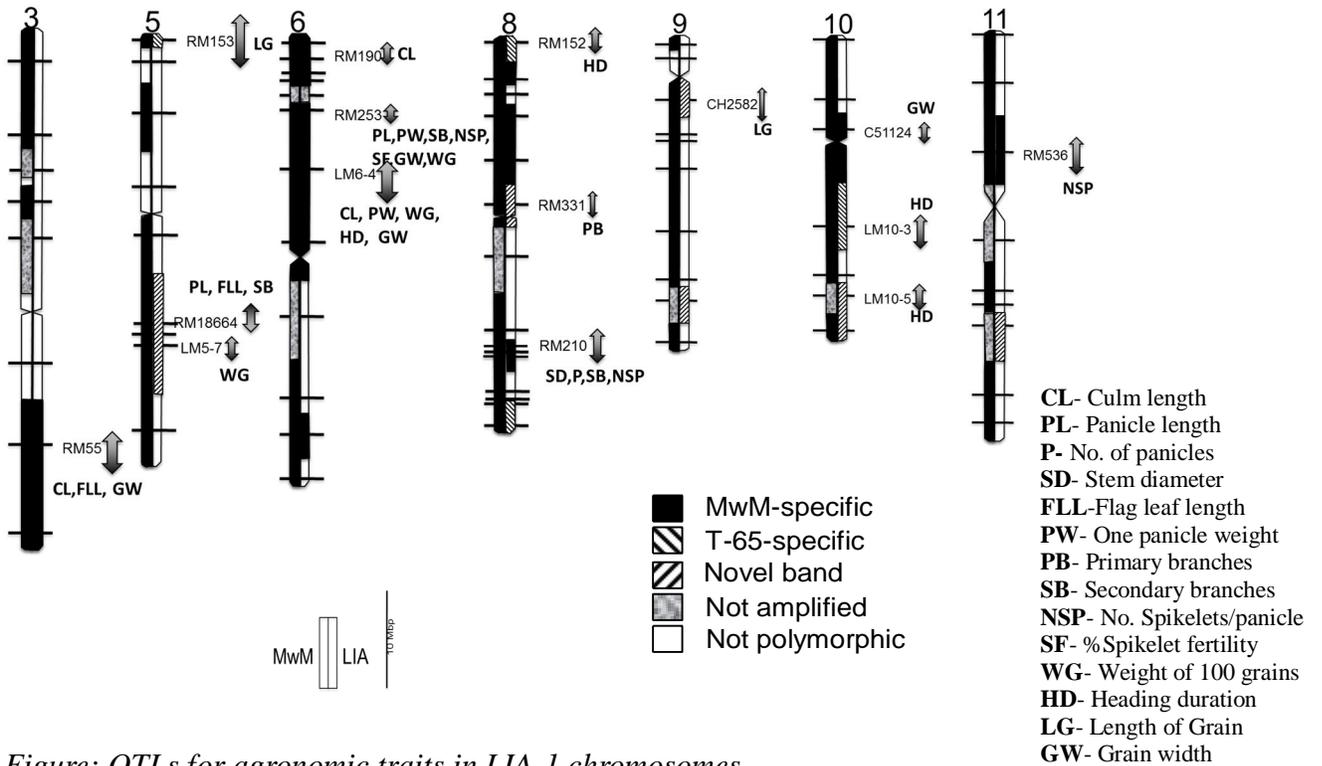*Figure 1: Polymorphic segments in MwM and LIA-1 compared to Norin 18*

Figure: QTLs for agronomic traits in LIA-1 chromosomes

CL- Culm length
PL- Panicle length
P- No. of panicles
SD- Stem diameter
FLL-Flag leaf length
PW- One panicle weight
PB- Primary branches
SB- Secondary branches
NSP- No. Spikelets/panicle
SF- %Spikelet fertility
WG- Weight of 100 grains
HD- Heading duration
LG- Length of Grain
GW- Grain width

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Table 1: Agronomic performance of LIA candidates under non-fertilized conditions

Line no.	No.plants	Culm length (cm)	Panicle length (cm)	No. panicles	Mean panicle weight (g)	No. primary branches	No. secondary branches	No. spikelets	SF (%)	100 grain weight (g)
11-362(LIA-1)	73	97.3	28.9	6	4.044	17.6	57.1	287.2	49.3	2.034
11-362(LIA-2)	10	118.1	29.6	6.8	7.107	18.4	82.9	403.3	73.5	2.354
11-363(LIA-3)	3	128.0	31.5	5.0	7.357	16.0	67.0	353.3	64.8	2.117
11-364(LIA-4)	10	94.7	29.5	5.0	5.690	18.6	67.4	403.3	46.9	2.373
11-365(LIA-5)	5	118.1	28.4	5.4	8.231	20.8	96.8	511.6	65.8	2.308
11-366(LIA-6)	3	124.2	29.2	7.0	6.554	16.3	69.0	388.0	77.2	2.355
Norin 18	21	83.4	21.8	14.5	2.614	13.1	20.9	135.7	94.7	2.210

Table2: Agronomic performance of Norin 18 and LIA-1 grown under fertilized and non-fertilized conditions

Condition	Line/Var.	No. plants	Culm length (cm)	Panicle length (cm)	No. panicles	Culm base diameter (mm)	Flag leaf length (cm)	Mean panicle weight (g)	No. primary branches	No. secondary branches	No. spikelets	SF%	100 grain weight (g)
Fertilized conditions	Norin 18	16	93.0	22.4	14.1	5.07	30.6	2.391	14.3	23.6	139.9	93.9	2.201
	LIA-1	16	108.8	30.4	6.1	8.24	39.4	3.289	16.7	55.8	272.2	49.6	2.133
Non-fertilized conditions	Norin 18	21	83.4	21.8	14.5	4.85	29.9	2.614	13.1	20.9	135.7	94.7	2.210
	LIA-1	73	97.3	28.9	6.0	8.75	41.0	4.044	17.6	57.1	287.2	49.3	2.034

Table3: Correlation among for yield related components in the 194 F2 plants of the cross between Norin 18 and LIA-1

Trait	Culm length (cm)	Panicle length (cm)	No. panicles	Stem diameter (mm)	one panicle weight (g)	No. primary branches	No. Secondary brances	No. spikelets	Spikelet fertility (%)	100 grains weight	Seed Length	Seed Width	Flag leaf length (cm)
Culm length (cm)	1												
Panicle length (cm)	-0.118	1											
No. panicles	0.230**	0.139	1										
Stem diameter(mm)	0.153	0.236**	0.059	1									
one panicle weight (g)	0.094	0.193*	0.017	0.347**	1								
No. primary branches	0.043	0.124	0.008	0.478**	0.267**	1							
No. Secondary brances	0.081	0.376**	0.167*	0.508**	0.502**	0.378**	1						
No. spikelets	0.071	0.320**	0.172*	0.520**	0.496**	0.533**	0.848**	1					
Spikelet fertility (%)	0.114	0.074	0.062	-0.007	0.545**	0.006	0.088	0.047	1				
100 grains weight	-0.224**	0.002	-0.021	-0.048	0.012	-0.028	-0.167*	-0.103	-0.266**	1			
Seed Length	-0.185*	-0.042	-0.033	-0.047	-0.09	-0.036	-0.168*	-0.180*	-0.089	0.155	1		
Seed Width	-0.054	-0.028	0.063	-0.027	-0.002	0.026	0.005	0.047	-0.089	0.146	-0.162*	1	
Flag leaf length (cm)	-0.307**	0.407**	-0.003	0.225**	0.154	0.248**	0.368**	0.349**	-0.105	0.052	0.016	0.12	1

*;** Significant at 0.05 and 0.01 levels, respectively.