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Identification of QTL for *Striga hermonthica* Resistance Using Backcross Population Derived from a Cross between *Oryza sativa* (cv. Nipponbare) and *O. rufipogon*

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Abstract

The obligate root hemiparasite, *Striga hermonthica* (Del.) Benth., native to sub-Saharan Africa causes serious economic constraint to cereal production. Studies on *Striga* spp. interactions with rice are desirable as it is a model monocot with high density molecular linkage maps. In this study, quantitative trait locus (QTL) analysis for *S. hermonthica* resistance was carried out using 141 backcross recombinant inbreed lines (BRILs) derived from a cross between *Oryza sativa* (cv. Nipponbare) and *O. rufipogon* W630. The population was grown in the field at Lake Basin Development Authority, Alupe farm in 2013 and infected with *S. hermonthica* from Alupe, Kenya. Putative QTL for *S. hermonthica* resistance was assumed using single-point analysis (qGene program) at $p < 0.01$ significance level. As a result, a single QTL explaining 6.6% of total phenotypic variance was detected near RM242 marker locus on chromosome 9, and the Nipponbare allele was found to have *S. hermonthica* resistance. The QTL chromosomal region can also be further studied to promote better understanding on the nature of resistance.

Keywords: quantitative trait loci (QTLs), rice, *Striga hermonthica*, resistance

1. Introduction

Rice is the most economically important food crop in sub-Saharan Africa (SSA). It is consumed widely in all countries and sub-regions of the continent and is mostly cultivated by resource-poor farmers under diverse ecosystems (Balasubramanian et al., 2007). Both cultivated rice species, *Oryza sativa* (L.) and *O. glaberrima* (Steud.), are grown in Africa. For the last 30 years, the harvested area has risen by 105% while production is by 170% (Rodenburg & Demont, 2009). However, the average rice production per unit is still low which is as a result of several production constraints, of which weed competition is regarded as the most severe. Weeds such as *Striga* spp. are the most problematic in upland conditions of SSA (Jamil et al., 2011). There are four *Striga* spp. considered to be serious pests to rice: *Striga hermonthica* (Del.) Benth., *S. asiatica* (L.) Kuntze, *S. aspera* (Willd.) Benth. and *S. forbesii* Benth. (Rodenburg et al., 2010; Atera et al., 2011). Among these species *S. hermonthica* is the most destructive, leading to severe yield losses of over 50% thereby affecting livelihoods of millions of farmers (Franke et al., 2006).

Cereal cultivars that offer resistance can post a major impact on limiting *Striga* infections and would dramatically improve yield (Rodenburg & Bastiaans, 2011). This would boost the morale of farmers in SSA as they view resistance as a desirable characteristic in cultivars. Several reports of resistance to *S. hermonthica* have been documented in rice (Harahap et al., 1993; Gurney et al., 2006; Jamil et al., 2011), sorghum [*Sorghum bicolor* (L.) Moench] (Vogler et al., 1996; Ezeaku & Gupta, 2004, Noubissie et al., 2012), pearl millet [*Pennisetum glaucum* (L.) R. Br.] (Kountche et al., 2013) and maize [*Zea mays* L.] (Amusan et al., 2008; Karaya et al., 2012). However, this information has not found its way to the hands of farmers as it is imbedded in books and journals which make it difficult for farmers to access.

In many studies, African rice species appears to offer better sources of resistance to *Striga* parasitism than Asian

rice species (Johnson et al., 2000; Kaewchumnong & Price, 2008). Harahap et al. (1993) and Johnson et al. (2000) reported resistance to *S. hermonthica* observed in African rice cultivars in CG14, IG10, Makassa and ACC102196, and in Asian rice cultivars IR49255-B-B-5-2 and IR47255-B-B-5-4. The inter-specific New Rice for Africa (NERICA) cultivars offers a potentially interesting gene pool of resistant rice cultivars. NERICA 1 and NERICA 10 are said to be resistant to *S. hermonthica* infections (Cissoko et al., 2011; Atera et al., 2012).

A resistant phenotype is characterized by lack of ability of the parasite to penetrate through the endodermis and therefore the parasite cannot make xylem-xylem connections to establish a vascular for continuity after attachment to the host. This has been demonstrated by Gurney et al. (2006) with Nipponbare, *O. sativa* Japonica lowland rice cultivar containing sources of resistance of post-attachment to *S. hermonthica*. Advanced backcross inbred lines (BILs) from a cross of Nipponbare and Kasalath (*O. sativa*, indica cultivar) were screened for resistance to *S. hermonthica* from Kibos, Kenya. Seven QTLs were detected on chromosomes 1, 4, 5, 6, 7, 8 and 12. Interestingly, Nipponbare conferred greater resistance allele in six out of the seven QTLs. The two largest QTLs (an indication where phenotypic variance in a population is greatest) were on chromosome 4 associated with Kasalath allele and chromosome 12 of Nipponbare.

In the study of Swarbrick et al. (2008) of Koshihikari-Kasalath BILs, three *Striga* resistance QTLs were detected, two of which were from Kasalath alleles with the largest located on chromosome 4. In addition, Kaewchumnong and Price (2008) identified two other QTLs on chromosomes 1 and 8 from the population derived from a cross between cultivars Bala and Azucena which coincided with the QTLs found by Gurney et al. (2006) for post-attachment resistance to *S. hermonthica* in Nipponbare and Kasalath population. The impact of *Striga* spp. infestation in farmer's cereal crop fields must be reduced at all cost. This can be done through rice as it has played a central role in human nutrition and culture for the past 10,000 years. In addition, rice has a small genome (c. 389Mb) which can easily be used for QTL mapping (International Rice Genome Sequencing Project, 2005). In this study, we detected *S. hermonthica* resistance QTL in rice using BRILs derived from a cross between *O. sativa* cv. Nipponbare and *O. rufipogon* W630.

2. Materials and Method

2.1 Site Description

The field study was conducted in the long rains of March to August 2013 at Alupe farm of Lake Basin Development Authority (LBDA), near Busia town (0°29′N, 34°07′E) in western Kenya, where *S. hermonthica* is a serious limitation to cereal crop production. The site is located at 1189m above sea level and normally receiving a mean annual rain and temperature of 1028 mm and 30 °C, respectively. Prior to the trial, the site was under cultivation of maize.

2.2 Plant Materials

In this study, *O. sativa* Japonica cultivar Nipponbare and a wild annual accession *O. rufipogon* W630 from Myanmar were used. *O. rufipogon* W630 is quite susceptible to *S. hermonthica* (although not as susceptible as other cultivars such as Dourado precoce) collected from Alupe, Kenya in a pre-test pot experiment conducted at Maseno University (Figure 1). Nipponbare had previously been classified as a resistant cultivar to *S. hermonthica* from Kibos, Kenya (Gurney et al., 2006). The segregating population for QTL analysis consisted of 141 backcross recombinant inbred lines (BRILs) between Nipponbare (a recurrent parent) and *O. rufipogon* W630 (a donor parent) at BC_2F_{10} generation. BRILs were obtained from Kobe University, Japan. The *S. hermonthica* seeds used in this study were collected from maize host in 2011 at Kenya Agricultural Research Institute (KARI) Alupe, Kenya.

2.3 Genome Composition and Phenotypic Recording of BRILs

Estimation of wild chromosomal segments in each of the 159 BRILs at BC2F8 generation ranged between 0.0 to 23.6% (Thanh et al., 2011). The estimation was done using 180 microsatellite loci covering 1,362 cM of the 12 rice chromosomes. The BRILs have about 11.3% of wild genome on average in the genetic background of Nipponbare and between three (3) to thirty nine (39) lines of the BRILs were identified to have wild homozygous alleles at these marker loci.

In this study, the 141 BRILs were each inoculated with 0.5 g of *S. hermonthica* seed per row as described by Berner et al. (1997). This weight of *Striga* seeds contained about 3000 germinable seeds per hill. Before inoculation, the *Striga* seeds were thoroughly mixed with fine soil which was sieved through a screen of pore diameter of 250 μm to serve as a carry since *Striga* seeds are very small. Each hill was 30 cm by 30 cm of which soil of about 10 cm diameter and depth of 5 cm was dug, and sprinkled with a scoop full of *Striga*-soil mixture. Three rice seeds of each BRIL were planted per hill in ten hills (already artificially inoculated with *Striga*) per

row. After germination, seedlings were thinned to one per hill. Fertilizer was applied at rate of 60 kg N ha-1. The infected field was weeded once with a hoe, after which the weeds were pulled by hand other than *Striga* to avoid damaging young *Striga* seedlings. Field data on the number of *S. hermonthica*, survived BRILs and infected hills per row were recorded.

A **B**

Figure 1. *S. hermonthica* infecting (A) *O. rufipogon* W630; (B) Dourado precoce, a highly susceptible cultivar in a pot experiment at Maseno University, Kenya

2.4 QTL Analysis

Based on the infected rates (no. infected plants / total no. plants examined) by *Striga* in the BRILs (giving more than five plants examined), QTL analysis was carried out. Single marker analysis (SMA) was used to estimate QTLs for *S. hermonthica* resistance by qGene software (Nelson, 1997). The significance threshold for SMA was set at $p < 0.01$ level. The proportion of phenotypic variation explained by significant marker was estimated as a coefficient of determination (R^2) for the single locus model.

3. Results and Discussion

3.1 Evaluation of Striga Resistance in Parents and BRILs

O. sativa Japonica cv. Nipponbare is resistance while wild accession *O. rufipogon* W630 was susceptible to *S. hermonthica* from Alupe, Kenya infections. From the pre-test pot experiment at Maseno University, Nipponbare had no *Striga* plants while *O. rufipogon* had at least two to four *Striga* per pot (Atera & Itoh, communication). In the field at Alupe, Nipponbare was attacked by very few *Striga* plants compared with the other cultivars. This confirmed the classification of Nipponbare as a resistant cultivar as described by several authors (Kaewchumnong & Price, 2008; Swarbrick et al., 2008). Unfortunately *O. rufipogon* being an aquatic species, it could not grow in upland conditions as germination was very poor. Field observations showed that 50.4% (71 out of 141) of the BRILs had no *Striga* infections based on the number of *Striga* attached to rice plants above the ground. The frequency distribution of infected plants by *S. hermonthica* is as shown in Figure 2. This result suggested that the resistance for *S. hermonthica* was under polygenic control.

3.2 Detection and Analysis of QTL

The threshold to declare a QTL was at the significance level of $p < 0.01$ as revealed by the genome scan (Figure 3). The QTL for *S. hermonthica* resistance was detected on chromosome 9 with a P value of 0.0022 and a PV value (percentage of phenotypic variance explained by the QTL) of 6.6% (Table 1). At $p < 0.05$ significance level, other loci having weak effects were detected on chromosomes 2, 3, 7 and 12 but they may not be considered biologically significant unless they are validated (Figure 3).

The result showed that *O. rufipogon* W630 allele at QTL on chromosome 9 increased the infection rate in the genetic background of Nipponbare. The Nipponbare-derived allele conferred resistance at this QTL as expected. In our QTL detection, the BRILs were subjected to *Striga* infections in its natural condition with several environmental interferences. Previous QTL detections for *S. hermonthica* resistance were post-attachment in cultivated rice and in a controlled environment (Gurney et al., 2006; Kaewchumnong & Price, 2008; Swarbrick et al., 2008). In field experiments, crop varieties interactions between *Striga* and its host determine the reproductive success of the parasite. For incidence, *Striga* seeds only germinate when exposed to moisture, a

favorable temperature and germination stimulant is required which is the host plant root exudates. These interactions sometimes provide opportunities to the host to resist the parasite.

Table 1. Putative QTL for *S. hermonthica* resistance detected in the back cross recombinant inbred lines derived from a cross between *O. sativa* Nipponbare and *O. rufipogon* W630

Chromosome	Marker	Source	P Value	[a]PV(%)	[b]Additive effect (%)
9	RM242	*O. rufipogon* W630	0.0022	6.6	7.6

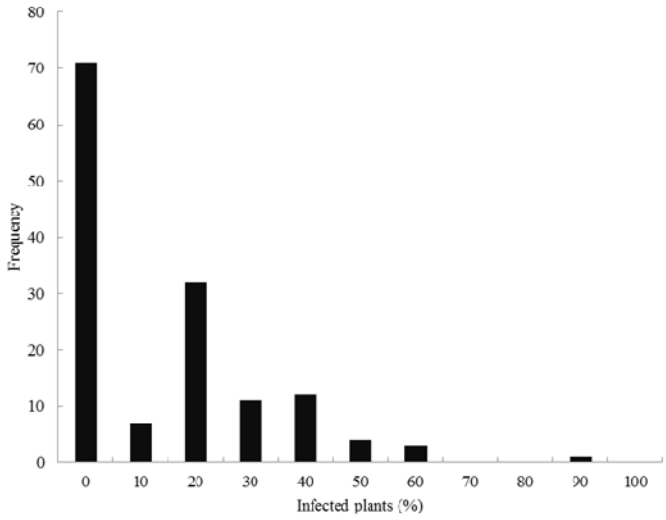

Figure 2. Frequency distribution of infected plants by *S. hermonthica* of the backcross recombinant inbred lines a cross between *O. sativa* Nipponbare and *O. rufipogon* W630. Frequency scores were calculated as the proportion of the lines in which *Striga* failed to attach to the BRILs

The detected major QTL can be further studied to see if there is a possibility of identifying candidates for resistance through fine mapping. It has been reported that genes that are expressed in infected plants tissues of two contrasting parents and able to map closely to a QTL are most likely to be appropriate candidates for host-resistance genes (Holloway et al., 2011). According to Swarbrick et al. (2008) resistance genes can be identified near the major QTL associated with resistance. If resistance genes can be identified in rice, orthologous genes may be estimated in other cereal crops such as maize and sorghum though the synteny of chromosomal gene order.

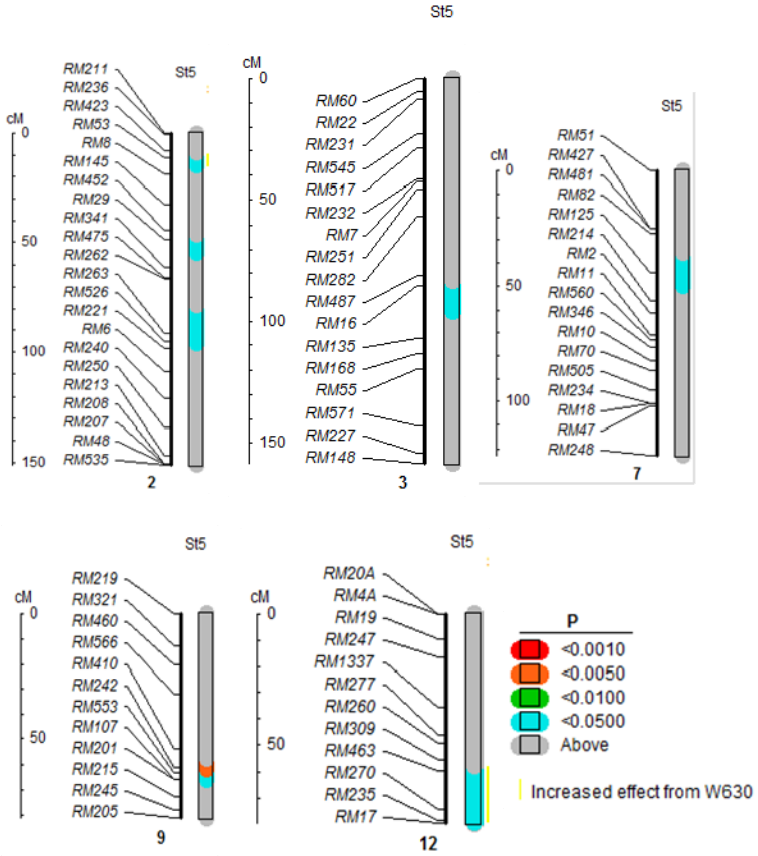

Figure 3. Genome scan of quantitative trait loci (QTL) for *Striga resistance* in *O. sativa* Nipponbare *and O. rufipogon* W630 population. The marker orders and the genomic regions associated with QTL scored at significance level at $P < 0.001$ and $P < 0.05$

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