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# Identification of QTLs for Agronomic Characteristics in An Upland New Rice for Africa (NERICA) Variety

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## Abstract

New Rice for Africa (NERICA) varieties have been developed by inter-specific crossings to produce rice suitable for the harsh environments in Africa. The basic idea of NERICA was to combine the most useful characteristics of an African species (*Oryza glaberrima*) and an Asian species (*Oryza sativa*). However, the genetic basis of the agronomic characteristics of NERICA varieties remains unknown. In a previous study, we detected QTLs for days to heading (DTH) by using a segregating population derived from a cross between NERICA10 and its parent variety of *O. sativa* (WAB56-104). In the present study, we evaluated the agronomic traits of the same population and found positive correlations between DTH and culm length, culm and leaf weight, and flag leaf length. Negative correlations were found between DTH and panicle weight (PW), and between the ratio of panicle weight per culm and leaf weight. In the QTL analysis, a total of 33 SSR markers showed significant association with more than one trait and some markers were associated with more than two traits, suggesting the presence of a QTL with pleiotropic effects on multiple traits or a cluster of QTLs controlling different traits. QTLs on chromosome 8 conferred shorter DTH and larger PW in NERICA10 than WAB56-104. This QTL might explain the negative correlation between DTH and PW in the population. This information on the QTLs of agronomic traits will be useful for improving NERICA varieties adapted to low-yielding environments in Africa.

**Discipline:** Plant breeding
**Additional key words:** days to heading, hybrid sterility, *Oryza glaberrima*, *Oryza sativa*

## Introduction

Global food demand is likely to increase with rapid population growth in the future. To meet the rising demand, rice production will also need to increase because rice is a staple crop for much of the world's population. Scientists of the West Africa Rice Development Association (later renamed the Africa Rice Center) have developed New Rice for Africa (NERICA) varieties adapted to low-yielding environments in west and central Africa (Jones et al. 1997a). The breeding of NERICA was initiated to combine such useful characteristics as weed competitiveness and disease tolerance of an African rice species (*Oryza glaberrima*) with the high yield potential of an Asian rice species (*Oryza sativa*) by using interspecific hybridization (Jones et al. 1997b, Sarla and Swamy 2005, Saito et al. 2012). By 2008, a total of 18 upland NERICA varieties had been released and were being grown widely in Africa (Africa Rice Center 2008). According to the Africa Rice Center (2008), NERICA varieties have high yield potential and a short growth cycle; these varieties also have resistance to local stressors such as rice blast disease and stem borers. Moreover, some NERICA varieties have biotic and abiotic stress tolerance (Cissoko et al. 2011, Kang et al. 2012, Oikeh et al. 2008). Despite these advantages, there is still limited knowledge about the genetic basis of such important traits. Recently, Saito et al. (2012) compared the upland NERICA varieties with their parent *O. sativa* (WAB56-104), and pointed out that upland NERICA varieties lack the expected combination of superior yield and weed competitiveness. These results indicated that current upland NERICA varieties still require improvements.

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Knowledge about the genes responsible for the agronomic characteristics of NERICA varieties will be valuable for further genetic improvement of the current upland NERICA varieties. In our previous comparison with 32 varieties of *O. sativa*, we characterized 18 upland NERICA varieties as heavy-panicle and low-tillering types with early heading (Fukuta et al. 2012). We also examined the association between the genotypes of those 18 NERICA varieties and their agronomic characteristics, and found QTLs at 61 simple sequence repeat (SSR) markers for 11 agronomic characteristics. Although that study provided useful information for the genetic improvement of upland NERICA varieties, we were unable to rule out the false-positive detection of QTLs due to the limited number of lines (18) used in the association analysis (Fukuta et al. 2012).

To rule out the false-positive detection of QTLs, we developed a segregating population using a cross between NERICA10 and one of its parent varieties, WAB56-104. Using this population, we successfully identified QTLs for days to heading (DTH) on chromosomes 4, 6, and 8 (Koide et al. 2015). Among these QTLs, *qDTH8.1*, located on chromosome 8, had the strongest effect. Although these results indicated the usefulness of the segregating population in detecting QTLs for characteristics that differ between NERICA10 and WAB56-104, the genetic basis of the other agronomic characteristics of NERICA10 remains unknown. Thus, in the present study, we identify QTLs for agronomic traits and examine the genetic associations between these traits and DTH using the segregating population.

## Materials and methods

### 1. Plant materials and field experiment

We used a total of 180 $F_2$ plants derived from crosses between NERICA10 and WAB56-104, and $F_3$ lines derived from self-pollination of the $F_2$ plants. These populations are the same as those we used previously for detecting QTLs for DTH (Koide et al. 2015). $F_2$ plants and $F_3$ lines were cultivated in a paddy field at the Japan International Research Center for Agricultural Sciences (JIRCAS) in Tsukuba, Ibaraki, Japan, from April to September in 2010 and 2011, respectively, together with their parent varieties. The mean day-length in the month of the cultivation period was 13.1 to 14.6 h. and considered a natural long day (NLD) condition. Three weeks after sowing, single plants were transplanted in the field using 18-cm spacing between hills and 30-cm spacing between rows. For each line, twelve plants were transplanted in one row. Cultivation management followed the standard procedures used at JIRCAS (Uddin et al. 2016).

We evaluated nine traits — panicle number per plant (PN), culm length (CL), panicle length (PL), panicle weight (PW), culm and leaf weight (CLW), ratio of panicle weight to culm and leaf weight (PW/CLW), flag leaf length (FLL) and flag leaf width (FLW) — in 2011, and evaluated seed fertility (SF) in 2010.

PN was counted as the number of productive panicles per plant. For the other traits, the tallest tiller of a plant was used for the measurement. CL was measured from the soil surface to the neck. PL was measured from the panicle neck to the panicle tip. PW was measured as the weight of the panicle. CLW was measured as the weight of the culm and leaves. PW/CLW was determined as the ratio of PW to CLW. FLL and FLW were measured for the flag leaf. SF was calculated by counting the number of filled and unfilled spikelets. SF was evaluated in the $F_2$ plants. The other eight traits were evaluated in the $F_3$ lines. In the $F_2$ plants, a single value per plant was measured for SF. In the $F_3$ lines, 10 individual plants in the middle of each row were sampled and an average value per line was determined for each trait.

### 2. QTL analysis

For the QTL analysis, we used genotype data of 52 SSR markers obtained in our previous study (Koide et al. 2015). QTL analysis was performed with QTL Cartographer software version 2.5 using single-marker regression analysis (Wang et al. 2011). A probability level of 0.01 was used as the threshold for significant associations between markers and traits.

## Results

### 1. Differences in agronomic characteristics between NERICA10 and WAB56-104 under field conditions

To examine the differences in agronomic characteristics between NERICA10 and WAB56-104 under field conditions, the two varieties were grown in a field in Tsukuba, Japan, and nine agronomic traits (PN, CL, PL, PW, CLW, PW/CLW, FLL, FLW and SF) were examined. Significant differences between NERICA10 and WAB56-104 were observed in six traits. NERICA10 had significantly lower values for CL, PW and CLW, and significantly higher values for PL, FLL and FLW than WAB56-104 (Table 1).

### 2. Variations in agronomic traits in $F_2$ and $F_3$ populations

To examine the genetic associations between agronomic traits, we analyzed nine agronomic traits (PN, CL, PL, PW, CLW, PW/CLW, FLL, FLW and SF) in the $F_2$ and $F_3$ populations (Fig. 1). In addition, we used DTH data from the results of Koide et al. (2015). Analysis of variance indicated that $F_2$ plants or $F_3$ lines showed significant genetic variation in all traits. Transgressive segregation was observed for all traits. Phenotypic correlation between the 10 traits showed that 22 combinations out of a possible 55

**Table 1. Agronomic characteristics of NERICA10 and WAB56-104**

| Variety | Averages of agronomic traits | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | DTH | PN | CL (cm) | PL (cm) | PW (g) | CLW (g) | PW/CLW | FLL (cm) | FLW (cm) | SF (%) |
| NERICA10 | 85.2±3.4** | 10.9±2.4 | 79.6±4.1** | 25.1±1.3** | 29.9±4.9** | 34.5±2.8** | 0.87±0.1 | 36.3±3.2** | 1.9±0.1** | 89.1±2.2 |
| WAB56-104 | 92.6±0.5 | 12.4±1.3 | 100.7±3.4 | 23.0±1.5 | 39.8±5.3 | 47.8±5.6 | 0.84±0.1 | 30.8±3.3 | 1.4±0.1 | 91.3±3.5 |

DTH: days to heading; PN: panicle number; CL: culm length; PL: panicle length; PW: panicle weight; CLW: culm and leaf weight; PW/CLW: panicle weight to culm and leaf weight ratio; FLL: flag leaf length; FLW: flag leaf width; SF: seed fertility.
** Significantly different from the value for WAB56-104 (paired t-test, $P < 0.01$).

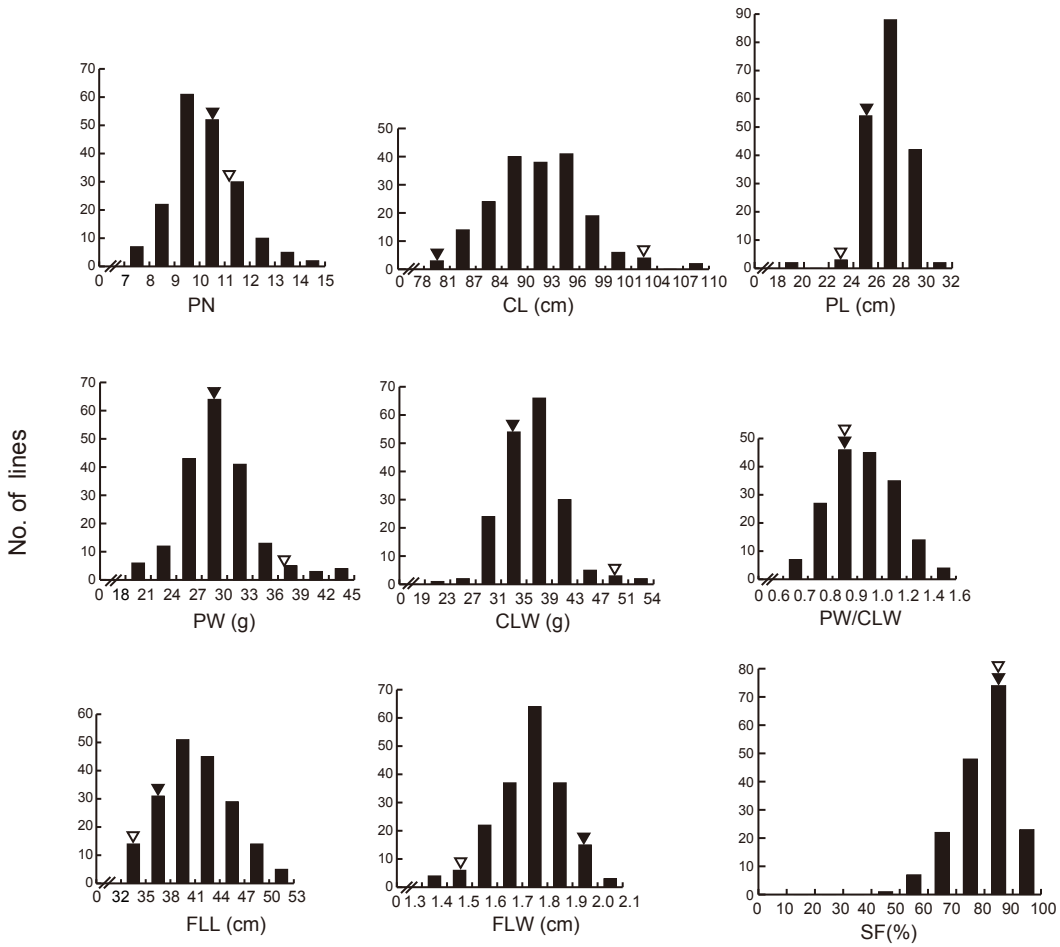


**Fig. 1 Frequency distributions of agronomic characteristics in segregating populations derived from crosses between NERICA10 and WAB56-104.**
Black and white arrows indicate mean values of the agronomic characteristics of NERICA10 and WAB56-104, respectively. PN: panicle number; CL: culm length; PL: panicle length; PW: panicle weight; CLW: culm and leaf weight; PW/CLW: ratio of panicle weight to culm and leaf weight; FLL: flag leaf length; FLW: flag leaf width; SF: seed fertility.

were significant (Table 2). DTH was positively correlated with CL, CLW and FLL, and negatively correlated with PW and PW/CLW.

## 3. QTL analysis for agronomic traits

We examined the chromosomal locations of QTLs for the nine agronomic traits other than DTH (PN, CL, PL, PW, CLW, PW/CLW, FLL, FLW and SF) using single-marker regression analysis. A total of 33 SSR markers showed significant associations with more than one trait (Fig. 2, Table 3). Some markers were associated with more than two traits. Such co-localization of QTLs for different ag-

**Table 2. Correlation coefficients among traits in the $F_2$ plants and $F_3$ lines derived from a cross between NERICA10 and WAB56-104**

| Trait | $F_2$ | $F_3$ | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | DTH | DTH | PN | CL | PL | PW | CLW | PW/CLW | FLL | FLW |
| $F_3$ | | | | | | | | | | |
| DTH | 0.75** | | | | | | | | | |
| PN | -0.15 | -0.25* | | | | | | | | |
| CL | 0.44** | 0.43** | 0.16 | | | | | | | |
| PL | -0.1 | -0.09 | 0.03 | -0.02 | | | | | | |
| PW | -0.31** | -0.47** | 0.58** | 0.15 | 0.36** | | | | | |
| CLW | 0.45** | 0.46** | 0.47** | 0.58** | 0.12 | 0.39** | | | | |
| PW/CLW | -0.69** | -0.83** | 0.12 | -0.36** | 0.23 | 0.57** | -0.52** | | | |
| FLL | 0.29** | 0.28** | -0.28** | -0.07 | 0.40** | 0.00 | 0.21 | -0.16 | | |
| FLW | 0.09 | 0.24 | -0.03 | 0.22 | 0.19 | 0.03 | 0.21 | -0.15 | 0.10 | |
| SF | 0.03 | -0.03 | 0.05 | -0.05 | 0.13 | 0.14 | 0.02 | 0.12 | 0.09 | 0.00 |

DTH: days to heading; PN: panicle number; CL: culm length; PL: panicle length; PW: panicle weight; CLW: culm and leaf weight; PW/CLW: panicle weight to culm and leaf weight ratio; FLL: flag leaf length; FLW: flag leaf width; SF: seed fertility.
* and ** Significant at $P = 0.05$ and 0.01, respectively, after Bonferroni correction.

ronomic characteristics was observed in five chromosomal regions: in the proximal and distal regions of the long arm of chromosome 4, on the short arm of chromosome 6, on the short arm of chromosome 8, and on the long arm of chromosome 9 (Fig. 2).

In the proximal region of the long arm of chromosome 4, one marker (RM7279) was associated with three agronomic traits — PN, FLL and FLW (Fig. 2). For this marker, the positive allele for PN and FLW came from NERICA10, whereas that for FLL came from WAB56-104. In the distal region of the long arm of chromosome 4, where *qDTH4.1* (Koide et al. 2015) is located, two markers (RM3276 and RM3843) were associated with two other traits (CL and FLW). For these markers, the positive allele for CL came from WAB56-104, whereas that for FLW came from NERICA10. On the short arm of chromosome 6, where *qDTH6.1* (Koide et al. 2015) is located, a total of nine markers showed significant association with SF and CLW. For these markers, the positive allele for SF came from NERICA10, whereas that for CLW came from WAB56-104. In addition, four markers (RM19359, RM19367, RM19387 and RM510) were significantly associated with PW/CLW. On the short arm of chromosome 8, where *qDTH8.1* (Koide et al. 2015) is located, a total of four markers were significantly associated with CL, CLW, PW, FLW and PW/CLW. For these markers, the positive allele for CL, CLW and FLW came from WAB56-104, whereas that for PW and PW/CLW came from NERICA10. On the long arm of chromosome 9, the marker RM1328 was associated with CL and FLW. For this marker, the positive alleles for CL and FLL came from NERICA10.

### 4. QTL analysis for hybrid sterility

In our QTL analysis, nine SSR markers on the short arm of chromosome 6 showed strong segregation ratio distortion (Table 4). The frequency of the NERICA10-derived allele was significantly higher than that expected from Mendelian inheritance. Among the nine markers, RM19359 had the strongest segregation ratio distortion ($P = 4.0 \times 10^{-90}$ in marker RM19359). The frequency of the NERICA10-derived allele was 90.0%, and there were no homozygotes for the WAB56-104-allele at RM19359 (Table 4). In addition, a QTL for SF was observed in this region. At marker RM19387, heterozygotes had significantly lower seed fertility (60.4%) than homozygotes for the parental allele (81.4%). These results suggested a QTL for hybrid sterility was located on the short arm of chromosome 6.

## Discussion

### 1. Co-localization of QTLs for agronomic traits

In a previous study, 18 upland NERICA varieties were compared with 32 different varieties of *O. sativa* in Tsukuba, Japan, and then categorized as short-DTH and heavy-panicle and low-tillering types (Fukuta et al. 2012). Koide et al. (2015) reported the presence of QTLs for short-

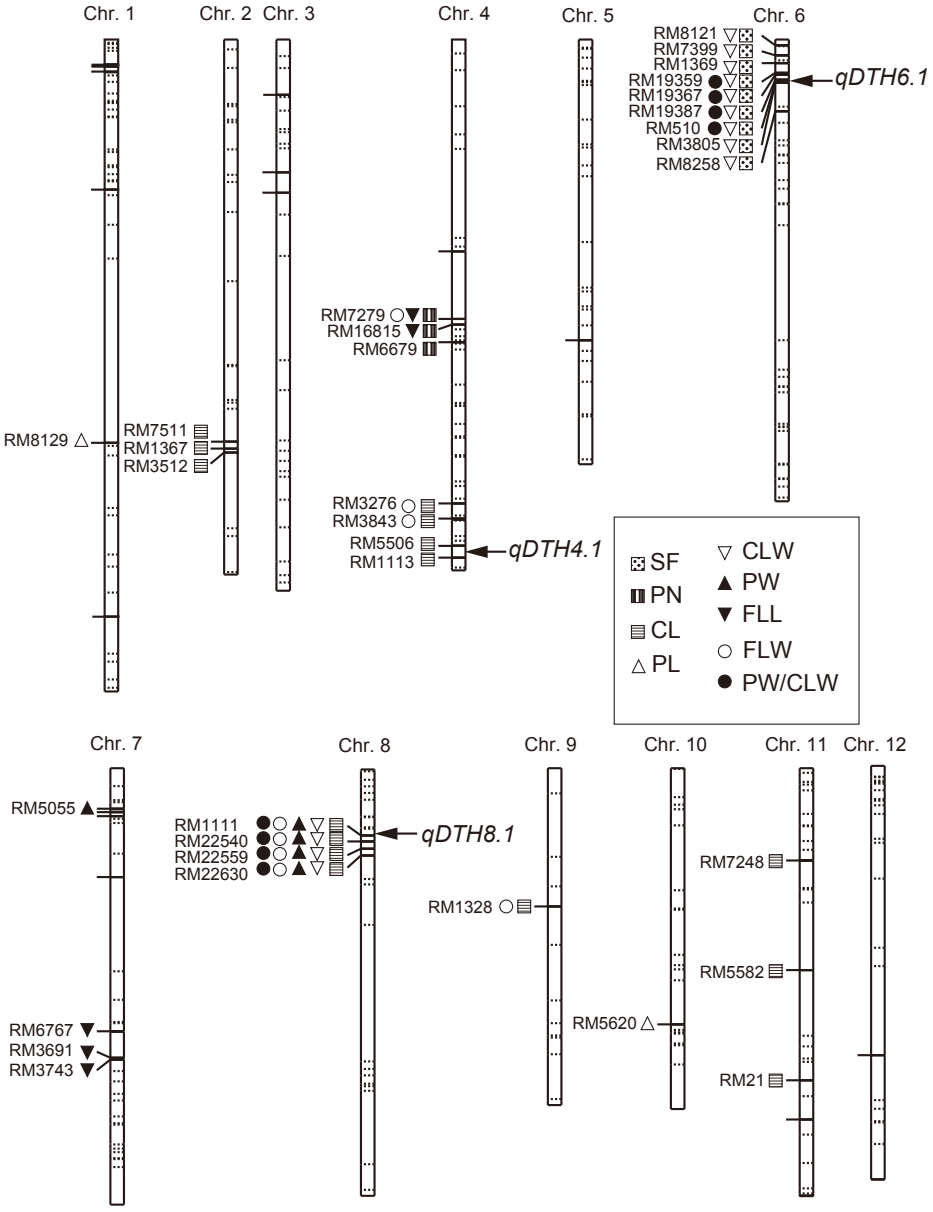


**Fig. 2 Chromosomal positions of markers associated with agronomic traits in NERICA10 × WAB56-104 $F_2$ and $F_3$ lines, and QTL regions detected in advanced QTL analysis.** Physical mapping positions of markers showing polymorphism between NERICA10 and WAB56-104 are indicated by horizontal bars on each chromosome. Physical mapping positions of non-polymorphic markers are shown by dotted bars. Markers showing significant associations in nine traits by single-marker regression analysis are shown on the left side of chromosomes. Chromosomal positions of QTLs for DTH detected in the previous study are shown by arrows on the right side of chromosomes.

DTH using the population derived from a cross between NERICA10 and WAB56-104. However, QTLs for the other agronomic characteristics have not been examined; consequently, the genetic basis of unique characteristics (i.e., short-DTH, heavy-panicle, low-tillering types) observed in NERICA remains unknown. In the present study, we performed QTL analysis for agronomic characteristics using $F_2$ and $F_3$ populations derived from a cross between NERICA10 and WAB56-104. NERICA10 had lower values for DTH, CL, PW and CLW, and higher values for PL, FLL and FLW than did WAB56-104. In the $F_3$ lines, phenotypic correlations were observed in 22 out of 55 possible com-

**Table 3. Markers associated with agronomic traits in the segregating population derived from a cross between NERICA10 and WAB56-104**

| Trait | Marker name | Chr. | Position (Mb)[a] | Positive allele[b] | $R^2$ | $F$ | $P$[c] | Additive effect |
|---|---|---|---|---|---|---|---|---|
| Panicle number (PN) | | | | | | | | |
| | RM7279 | 4 | 14.0 | NE10 | 0.02 | 9.0 | ** | 0.42 |
| | RM16815 | 4 | 18.5 | NE10 | 0.10 | 20.0 | **** | 0.56 |
| | RM6679 | 4 | 19.9 | NE10 | 0.05 | 9.2 | ** | 0.39 |
| Culm length (CL) | | | | | | | | |
| | RM7511 | 2 | 26.6 | NE10 | 0.07 | 14.7 | *** | 1.88 |
| | RM1367 | 2 | 27.1 | NE10 | 0.08 | 15.7 | *** | 2.00 |
| | RM3512 | 2 | 27.3 | NE10 | 0.06 | 14.7 | *** | 1.94 |
| | RM3276 | 4 | 30.7 | WAB | 0.09 | 17.2 | **** | 2.11 |
| | RM3843 | 4 | 31.7 | WAB | 0.12 | 23.5 | **** | 2.62 |
| | RM5506 | 4 | 33.5 | WAB | 0.12 | 23.7 | **** | 2.49 |
| | RM1113 | 4 | 34.3 | WAB | 0.11 | 22.4 | **** | 2.46 |
| | RM1111 | 8 | 4.8 | WAB | 0.13 | 27.0 | **** | 2.71 |
| | RM22540 | 8 | 5.3 | WAB | 0.16 | 31.4 | **** | 2.79 |
| | RM22559 | 8 | 5.7 | WAB | 0.10 | 20.9 | **** | 2.46 |
| | RM22630 | 8 | 7.3 | WAB | 0.17 | 35.7 | **** | 3.12 |
| | RM1328 | 9 | 9.2 | NE10 | 0.10 | 18.7 | **** | 2.21 |
| | RM7248 | 11 | 7.9 | WAB | 0.06 | 11.6 | *** | 1.60 |
| | RM5582 | 11 | 17.7 | WAB | 0.08 | 14.8 | *** | 2.20 |
| | RM21 | 11 | 21.7 | WAB | 0.08 | 14.9 | *** | 2.13 |
| Panicle length (PL) | | | | | | | | |
| | RM8129 | 1 | 26.7 | NE10 | 0.04 | 8.3 | ** | 0.51 |
| | RM5620 | 10 | 17.0 | NE10 | 0.07 | 13.8 | *** | 0.60 |
| Panicle weight (PW) | | | | | | | | |
| | RM5055 | 7 | 2.7 | WAB | 0.04 | 9.1 | ** | 1.27 |
| | RM1111 | 8 | 4.8 | NE10 | 0.08 | 14.7 | *** | 1.62 |
| | RM22540 | 8 | 5.3 | NE10 | 0.06 | 11.6 | *** | 1.40 |
| | RM22559 | 8 | 5.7 | NE10 | 0.06 | 12.2 | *** | 1.52 |
| | RM22630 | 8 | 7.3 | NE10 | 0.06 | 11.0 | ** | 1.45 |
| Culm and leaf weight (CLW) | | | | | | | | |
| | RM8121 | 6 | 0.4 | WAB | 0.03 | 12.8 | *** | 3.49 |
| | RM7399 | 6 | 1.0 | WAB | 0.08 | 16.0 | **** | 4.08 |
| | RM1369 | 6 | 1.6 | WAB | 0.08 | 15.9 | **** | 4.07 |
| | RM19359 | 6 | 2.2 | WAB | 0.08 | 16.2 | **** | 4.38 |
| | RM19367 | 6 | 2.3 | WAB | 0.07 | 13.9 | *** | 3.98 |
| | RM19387 | 6 | 2.7 | WAB | 0.09 | 15.9 | **** | 4.15 |
| | RM510 | 6 | 2.8 | WAB | 0.08 | 15.2 | *** | 3.89 |
| | RM3805 | 6 | 2.9 | WAB | 0.02 | 10.7 | ** | 3.13 |
| | RM8258 | 6 | 4.7 | WAB | 0.04 | 11.8 | *** | 3.10 |
| | RM1111 | 8 | 4.8 | WAB | 0.21 | 46.3 | **** | 3.09 |
| | RM22540 | 8 | 5.3 | WAB | 0.23 | 54.6 | **** | 3.18 |
| | RM22559 | 8 | 5.7 | WAB | 0.19 | 40.6 | **** | 2.98 |
| | RM22630 | 8 | 7.3 | WAB | 0.21 | 48.1 | **** | 3.21 |
| Panicle weight to culm and leaf weight ratio (PW/CLW) | | | | | | | | |
| | RM19359 | 6 | 2.2 | NE10 | 0.05 | 8.6 | ** | 0.10 |
| | RM19367 | 6 | 2.3 | NE10 | 0.05 | 8.4 | ** | 0.10 |
| | RM19387 | 6 | 2.7 | NE10 | 0.05 | 9.4 | ** | 0.10 |
| | RM510 | 6 | 2.8 | NE10 | 0.04 | 7.8 | ** | 0.09 |
| | RM1111 | 8 | 4.8 | NE10 | 0.41 | 120.0 | **** | 0.13 |
| | RM22540 | 8 | 5.3 | NE10 | 0.41 | 124.3 | **** | 0.13 |
| | RM22559 | 8 | 5.7 | NE10 | 0.34 | 92.4 | **** | 0.12 |
| | RM22630 | 8 | 7.3 | NE10 | 0.36 | 100.0 | **** | 0.13 |
| Flag leaf length (FLL) | | | | | | | | |
| | RM7279 | 4 | 14.0 | WAB | 0.02 | 7.7 | ** | 1.26 |
| | RM16815 | 4 | 18.5 | WAB | 0.07 | 11.3 | *** | 1.39 |
| | RM6767 | 7 | 17.4 | NE10 | 0.08 | 16.3 | **** | 1.64 |
| | RM3691 | 7 | 19.2 | NE10 | 0.07 | 13.6 | *** | 1.51 |
| | RM3743 | 7 | 19.3 | NE10 | 0.08 | 14.3 | *** | 1.54 |
| Flag leaf width (FLW) | | | | | | | | |
| | RM7279 | 4 | 14.0 | NE10 | 0.04 | 14.3 | *** | 0.05 |
| | RM3276 | 4 | 30.7 | WAB | 0.05 | 10.0 | ** | 0.04 |
| | RM3843 | 4 | 31.7 | WAB | 0.06 | 10.5 | ** | 0.05 |
| | RM1111 | 8 | 4.8 | WAB | 0.10 | 18.8 | **** | 0.06 |
| | RM22540 | 8 | 5.3 | WAB | 0.09 | 18.3 | **** | 0.06 |
| | RM22559 | 8 | 5.7 | WAB | 0.05 | 9.0 | ** | 0.04 |
| | RM22630 | 8 | 7.3 | WAB | 0.05 | 9.1 | ** | 0.05 |
| | RM1328 | 9 | 9.2 | WAB | 0.06 | 11.3 | *** | 0.05 |
| Seed fertility (SF) | | | | | | | | |
| | RM8121 | 6 | 0.4 | NE10 | 0.15 | 87.4 | **** | 0.17 |
| | RM7399 | 6 | 1.0 | NE10 | 0.37 | 110.3 | **** | 0.19 |
| | RM1369 | 6 | 1.6 | NE10 | 0.37 | 109.8 | **** | 0.19 |
| | RM19359 | 6 | 2.2 | NE10 | 0.46 | 151.9 | **** | 0.23 |
| | RM19367 | 6 | 2.3 | NE10 | 0.41 | 124.3 | **** | 0.21 |
| | RM19387 | 6 | 2.7 | NE10 | 0.39 | 113.4 | **** | 0.20 |
| | RM510 | 6 | 2.8 | NE10 | 0.35 | 96.9 | **** | 0.19 |
| | RM3805 | 6 | 2.9 | NE10 | 0.32 | 89.3 | **** | 0.17 |
| | RM8258 | 6 | 4.7 | NE10 | 0.16 | 52.7 | **** | 0.13 |

Associations between markers and traits were detected by single-marker regression analysis.

[a] Chromosomal positions are based on the Nipponbare genome sequence.

[b] NE10 and WAB indicate alleles of NERICA10 and WAB56-104 types, respectively.

[c] **, *** and **** Significance at 1%, 0.1%, and 0.01% levels, respectively.

**Table 4. Markers showing segregation ratio distortion**

| Marker name | Chr. | Position (Mb) | Genotype %[a] | | | $\chi^2$ | *P* |
|---|---|---|---|---|---|---|---|
| | | | N/N | N/W | W/W | | |
| RM8121 | 6 | 0.38 | 0.86 | 0.14 | 0.00 | 300.22 | $6.4x10^{-66}$ |
| RM7399 | 6 | 0.44 | 0.89 | 0.11 | 0.00 | 373.67 | $7.2x10^{-82}$ |
| RM1369 | 6 | 1.56 | 0.89 | 0.11 | 0.00 | 373.67 | $7.2x10^{-82}$ |
| RM19359 | 6 | 2.20 | 0.91 | 0.09 | 0.00 | 411.68 | $4.0x10^{-90}$ |
| RM19367 | 6 | 2.34 | 0.89 | 0.11 | 0.00 | 397.10 | $5.8x10^{-87}$ |
| RM19387 | 6 | 2.66 | 0.89 | 0.11 | 0.00 | 387.48 | $7.3x10^{-85}$ |
| RM510 | 6 | 2.83 | 0.88 | 0.12 | 0.00 | 372.87 | $1.1x10^{-81}$ |
| RM3805 | 6 | 2.85 | 0.89 | 0.11 | 0.00 | 359.05 | $1.1x10^{-78}$ |
| RM8258 | 6 | 4.44 | 0.87 | 0.13 | 0.00 | 330.82 | $1.5x10^{-72}$ |

[a] NN and NW: NERICA10-derived allele homozygote and heterozygote, respectively; WW: WAB56-104-derived allele homozygote

binations among the 10 agronomic traits. In rice, a longer duration of vegetative growth often increases biomass and grain yield (Thomson et al. 2003, Fujita et al. 2009). We found that DTH was positively correlated with CL, CLW and FLL, and negatively correlated with PW and PW/CLW, suggesting the presence of genes that confer short DTH and high PW simultaneously in NERICA10. Understanding the genetic basis of the unique association between negative DTH and positive PW is important for breeding varieties with high harvest indexes over short growth periods. To obtain more detailed knowledge about the genetic basis of such a unique association, genetically fixed lines such as recombinant inbred lines must be used.

The phenotypic association between traits observed in the $F_3$ lines implies the presence of a QTL with pleiotropic effects on multiple traits or, alternatively, a cluster of QTLs controlling different traits (Onishi et al. 2007, Bai et al. 2010). In our QTL analysis, we detected co-localization of QTLs for different traits in five chromosomal regions: in the proximal and distal regions of the long arm of chromosome 4, on the short arm of chromosome 6, on the short arm of chromosome 8, and on the long arm of chromosome 9 (Fig. 2). Among the five chromosomal regions, co-localizations of QTLs for agronomic traits and DTH (*qDTH4.1*, *qDTH6.1* and *qDTH8.1*) were observed in three regions. Several studies have shown that the genes for DTH control other yield-related traits (Xue et al. 2008, Wei et al. 2010, Endo-Higashi and Izawa 2011). Xue et al. (2008) reported that *Ghd7* controlling DTH affects the grain number. In addition, Endo-Higashi and Izawa (2011) showed that *Hd1* and *Ehd1* were able to control panicle development in rice. Our results agree that some agronomic characteristics are controlled by the QTL for DTH, although we were unable to rule out the possibility of the presence of QTL clusters for different traits in these regions.

Among the three QTL regions for DTH, the markers near *qDTH8.1* showed the highest *F* values for CL, CLW, PW, PW/CLW and FLW in the QTL analysis using the $F_3$ lines (Table 2). Koide et al. (2015) revealed that NERICA10 has the non-functional allele of the gene, *DTH8*, located in the region of *qDTH8.1* and suggested that the non-functional allele is the causal factor of *qDTH8.1*. Wei et al. (2010) revealed that a line with a non-functional *DTH8* allele had an earlier heading date, shorter plant height, and less dry weight. In the present study, the negative allele for DTH, CL, CLW and FLW came from NERICA10, corresponding with the finding of Wei et al. (2010). However, the negative allele for PW came from WAB56-104. Wei et al. (2010) reported that the presence of a functional *DTH8* allele increased the number of grains per panicle. Such discrepancies might be explained by differences in genetic background or environment, or both. We therefore need to develop near-isogenic lines of *qDTH8.1* and confirm the effect of *qDTH8.1* on PW under various conditions. Although the underlying molecular mechanisms are still unknown, co-localization of *qDTH8.1* and the QTL for PW would explain our finding that DTH was negatively correlated with PW and PW/CLW in the $F_3$ lines.

Since we used the population from a cross between NERICA10 and WAB56-104, NERICA10 was developed from a cross between CG 14 (*Oryza glaberrima*) and WAB56-104, alleles of the QTLs in NERICA10 detected in the present study were considered as being derived from CG 14. However, Koide et al. (2015) revealed that neither

WAB56-104 nor CG 14 had the same non-functional allele as NERICA10 in the *DTH8* locus. This result suggested the presence of non-parental introgression during the process of development of NERICA or polymorphism within the parental line (CG 14).

### 2. QTL for hybrid sterility in NERICA10

There are strong reproductive barriers in the interspecific crossing of *O. sativa* with *O. glaberrima*: hybrids show pollen sterility as well as seed sterility (Sano 1990, Doi et al. 1998, Heuer and Miézan 2003). Ikeda et al. (2009) revealed that $F_1$ hybrids between NERICA varieties and *O. glaberrima* are highly sterile. In addition, the $F_1$ hybrids between some NERICA varieties and *O. sativa* show semi-sterility (Ikeda et al. 2009), making it difficult to improve NERICA varieties using conventional breeding processes.

Using DNA markers to select fertile plants and remove genes causing sterility early in the breeding process is one approach to overcoming hybrid sterility. Our results showed the presence of a QTL for SF on the short arm of chromosome 6. In this chromosomal region, the hybrid sterility gene *S1*, which causes both pollen and seed sterility in the heterozygote has been identified in a cross between *O. sativa* and *O. glaberrima* (Sano 1990, Koide et al. 2008, Garavito et al. 2010). The *S1* allele, which is derived from *O. glaberrima*, causes preferential dysfunction of male and female gametes carrying its opposite allele ($S1^a$) derived from *O. sativa* in heterozygotes ($S1/S1^a$). Thus, segregation ratio distortion is observed in later generations of heterozygotes (Sano 1990, Koide et al. 2008, Garavito et al. 2010). In our study, at marker RM19387 in this QTL region, heterozygotes had significantly lower seed fertility (60.4%) than homozygotes for the parental allele (81.4%). We also found segregation ratio distortion at the markers on the short arm of chromosome 6.

These results strongly suggest the presence of *S1* in NERICA10. Our findings that there were no QTLs for SF and no markers showing significant segregation ratio distortion, except in the chromosomal region near *S1*, suggest that *S1* is the main genetic factor causing hybrid sterility in crosses between NERICA10 and WAB56-104. These findings, together with the information revealed by Koide et al. (2008) on the position of *S1*, will facilitate the development of DNA markers for selecting out hybrid sterility genes in NERICA varieties.

## Conclusions

We identified QTLs for agronomic traits using segregating populations derived from a NERICA10 × WAB56-104 cross under field conditions. Co-localization of QTLs for different traits were observed in five chromosomal regions. Among the five, three QTLs are co-localized with those for DTH that we previously identified. QTL for PW was co-localized with the previously identified QTL for DTH (*qDTH8.1*), suggesting the presence of a pleiotropic QTL or a cluster of QTLs that controls unique agronomic characteristics in NERICA. Moreover, we identified QTL for SF on the short arm of chromosome 6. This information will prove useful in improving NERICA varieties adapted to local environments in Africa.

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