Research Paper

Fine mapping of a major quantitative trait locus, \( qgnp7(t) \), controlling grain number per panicle in African rice (\( Oryza glaberrima \) S.)

Zejun Hu\(^{1,2} \), Liming Cao\(^{3} \), Xuejun Sun\(^{1,2} \), Yu Zhu\(^{2} \), Tianyu Zhang\(^{4} \), Lin Jiang\(^{2} \), Yahui Liu\(^{2} \), Siqing Dong\(^{2} \), Dayun Sun\(^{2} \), Jingshui Yang\(^{2} \), Haohua He\(^{1} \) and Xiaojin Luo*\(^{2} \)

\(^{1} \) Group of Crop Genetics and Breeding, Jiangxi Agricultural University, Nanchang 330045, China
\(^{2} \) State Key Laboratory of Genetic Engineering, Institute of Genetics, School of Life Sciences, Fudan University, Shanghai 200433, China
\(^{3} \) Institute of Crop Breeding and Cultivation, Shanghai Academy of Agricultural Sciences, Shanghai 201403, China
\(^{4} \) Deng Jia Bu Rice Foundation Seed Farm of Jiangxi Province, Yujiang 335200, China

Grain number per panicle is a major component of rice yield that is typically controlled by many quantitative trait loci (QTLs). The identification of genes controlling grain number per panicle in rice would be valuable for the breeding of high-yielding rice. The \( Oryza glaberrima \) chromosome segment substitution line 9IL188 had significantly smaller panicles compared with the recurrent parent 9311. QTL analysis in an F\(_2\) population derived from a cross between 9IL188 and 9311 revealed that \( qgnp7(t) \), a major QTL located on the short arm of chromosome 7, was responsible for this phenotypic variation. Fine mapping was conducted using a large F\(_3\) population containing 2250 individuals that were derived from the F\(_2\) heterozygous plants. Additionally, plant height, panicle length, and grain number per panicle of the key F\(_4\) recombinant families were examined. Through two-step substitution mapping, \( qgnp7(t) \) was finally localized to a 41 kb interval in which eight annotated genes were identified according to available sequence annotation databases. Phenotypic evaluation of near isogenic lines (NIL-\( qgnp7 \) and NIL-\( qGNP7 \)) indicated that \( qgnp7(t) \) has pleiotropic effects on rice plant architecture and panicle structure. In addition, yield estimation of NILs indicated that \( qGNP7(t) \) derived from 9311 is the favorable allele. Our results provide a foundation for isolating \( qgnp7(t) \). Markers flanking this QTL will be a useful tool for the marker-assisted selection of favorable alleles in \( O. glaberrima \) improvement programs.

Key Words: African rice (\( Oryza glaberrima \) S.), grain number per panicle, chromosome segment substituted lines, fine mapping.

Introduction

Rice (\( Oryza sativa \) L.) is one of the most important grain crops worldwide with increasing rice per unit yield and total yield playing an important role in ensuring global food security. Three components largely determine rice yield: effective tillers per plant, grain number per panicle, and grain weight (Sakamoto and Matsuoka 2008). As with the other components, grain number per panicle is controlled by many quantitative trait loci (QTLs) and is affected by environmental factors. As grain number per panicle is a typical quantitative trait, characterizing it using conventional methods is difficult. Molecular markers are a useful tool for investigating the genetic basis of complex quantitative traits (Kurata et al. 1994, McCouch et al. 1988, 2002). With QTL analysis based on molecular markers, grain yield has been extensively investigated and numerous QTLs have been reported over the last decade. According to the Gramene database information, there are 353 QTLs associated with grain number per panicle (www.gramene.org). Most of the materials used in mapping were, however, primary segregating populations such as F\(_2\), recombinant inbred lines (RILs), and doubled haploid lines (DHs). The excessive genetic background noise in these lines means that results

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*Corresponding author (e-mail: luoxj@fudan.edu.cn)
† These authors contributed equally to this work

Abbreviations: CSSL: chromosome segment substitution line; DHs: doubled haploid lines; IN1-IN6: first-six internode from top; Indel: insertion/deletion; GPP: grains per panicle; LOD: log of odds; NILs: near-isogenic lines; PCR: polymerase chain reaction; PB: primary branch; PH: plant height; PL: panicle length; QTL: quantitative trait locus; RILs: recombinant inbred lines; SB: secondary branch; SSR: simple sequence repeat
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obtained from each are variable and there is limited comparability among lines, because background genes can suppress or exacerbate phenotypes which are ubiquitous and highly polymorphic.

For fine mapping of a QTL, the effect of genetic background on the expression of the QTL should be eliminated. Thus, advanced populations such as chromosome segment substituted lines (CSSLs) and near-isogenic lines (NILs) containing one or a small number of introgressed fragments from a donor parent into a recipient parent genetic background are ideal materials for the fine mapping and isolation of target QTLs (Howell et al. 1996). Secondary segregation populations, which include F2 and F3 populations developed from a cross between one NIL with the target QTL and the recurrent parent, can be used to identify recombinants with the introgressed segment using flanking markers. Based on this strategy, several QTLs for rice grain number per panicle have been finely mapped (Chen et al. 2014, Deshmukh et al. 2010, Liu et al. 2009, Xie et al. 2008, Xing et al. 2008) and even isolated in recent years (Ashikari et al. 2005, Bai et al. 2010, Huang et al. 2009, Huo et al. 2017, Song et al. 2007, Wu et al. 2016, Xue et al. 2008, Zhao et al. 2015).

In this study, we report our research on the quantitative trait locus, qgnp7(t) for grain number per panicle on chromosome 7. A CSSL, 9IL188, with O. glaberrima chromosome segments in the indica rice 9311 background, showed significantly smaller panicles containing fewer grains compared with the recurrent parent 9311. We found that the qgnp7(t) allele from O. glaberrima was responsible for this variation. We then carried out fine mapping and localized qgnp7(t) to a 41 kb region on the short arm of chromosome 7. These results provide a foundation for isolating qgnp7(t) and investigating its molecular mechanism.

Materials and Methods

Plant materials and growth conditions

9IL188 is a CSSL developed by introgressing chromosome segments from a cultivar of African cultivated rice (IRGC102305, O. glaberrima) into Asian Oryza sativa 9311 background based on four generations of backcrossing and four generations of selfing. A small F2 population consisting of 152 plants derived from 9IL188 × 9311 was grown in a paddy field in Sanya (109°E, 18°N), Hainan Province, China, in the winter of 2013. Heterozygous F2 plants in the target region were selected to produce a larger F3 population for fine mapping of qgnp7(t). The F3 population containing 2,258 plants was grown in a paddy field in Taicang (121°E, 31°N), Jiangsu province, China, in the summer of 2014. The key F4 recombinant lines derived from the F3 population were grown in Sanya as above and used for progeny testing.

Phenotypic evaluation

Quantitative analysis of agronomic traits including plant height, tillers per plant, grains per panicle, seed setting rate, and 1,000-grain weight for the two parents, 9IL188 and 9311, were performed using 10 plants. The number of grains per panicle was evaluated for the 152 plants of the F2 population by dividing total number of grains per plant by tillers per plant. Quantitative analysis of plant height and grains per panicle for the two parents and the key recombinant lines derived from the F3 population were performed using 30 plants each. Quantitative analysis of agronomic traits including plant height, first-six internode from top (IN1–IN6), panicle length, number of primary and secondary branches per panicle (PB and SB), grains per panicle, seed setting rate, 1,000-grain weight, tillers per plant, and grain yield per plant for the two NILs, NIL-qgnp7 and NIL-qGNP7, were performed using 10 plants.

DNA extraction and molecular marker analysis

Micro-quantities of DNA were extracted from fresh rice leaves following a published method (Murray and Thompson 1980) with minor modification. Polymerase chain reaction (PCR) amplification was performed as previously described (Panaud et al. 1996). PCR products were separated by 6% polyacrylamide gel electrophoresis and detected by silver staining (Ji et al. 2007). Simple sequence repeat (SSR) markers in the qgnp7(t) region were identified from the Gramene database for grasses (http://www.gramene.org/). Besides the public molecular markers, four insertion/deletion (indel) markers containing polymorphisms between 9IL188 and 9311 were newly developed according to the publicly available rice genome sequence (http://www.gramene.org/) for fine mapping. The primer information for the molecular markers used in substitution mapping is listed in Table 1.

Data analysis

Likelihood ratio chi-square test was used to test the degree of significance, and the correlation degree was detected by P value in SPSS v17.0. Mapmaker/Exp v3.0 (Lincoln et al. 1992) was used for linkage analysis based on the 157-plant F2 population. The Kosambi function was used to calculate the genetic distance. Map Manager QTXb17 (Manly et al. 2001) was used to determine the QTL positions, the expected additive and dominance effects, and the phenotypic variance explained by individual QTLs. The genotypes of all markers in the target region were determined for the recombinant plants in the F3 family and were further confirmed by progeny testing.

Sequence analysis of candidate genes

Full genomes of Oryza sativa indica and Oryza glaberrima were downloaded from the website: http://www.gramene.org/, and full genome of Oryza glaberrima was also sequenced using Illumina/Solexa technology by BGI (Beijing Genome Institute, China). Candidate genes in the target region were amplified from 9311 and O. glaberrima using PCR with LA-Taq polymerase (Takara, Otsu, Japan). PCR products were purified with a PCR purification kit (Axygen, USA), introduced into a pGEM-T Easy Vector (Promega,
A total of 326 SSR markers, which are polymorphic between the two parents and evenly distributed on 12 chromosomes, were used to detect the substitution sites in 9IL188. Six *O. glaberrima* chromosome segments of approximately 55 cM in total were detected in the 9311 background and were located on chromosomes 1, 6, 7, and 12 (Fig. 2). The 152 plants of the F2 population were phenotyped for GPP at the grain filling stage and genotyped using 10 SSR markers distributed on the six substitution sites. Of these, there were 33 plants showing small panicles (<100 spikeles) and dwarfing (<80 cm). Progeny testing further confirmed that 46 and 44 families showed identical small and large panicles, respectively, whereas 62 families showed varied panicles (Fig. 3). The segregation ratio agreed with the Mendelian ratio ($\chi^2 = 4.67 < \chi^2_{0.05, 2} = 5.99$). These data indicated that the phenotypic difference between 9IL188 and 9311 was mainly controlled by a single gene. QTL analysis revealed that one QTL with a LOD value of 64.21 for GPP was located in the *O. glaberrima* interval between RM298 and RM82 on chromosome 7. We tentatively named this QTL qgnp7(t) and preliminarily mapped it to a 6.0 cM region between SSR markers RM298 and RM82 (Fig. 4A). The genetic distances from the target gene to two SSR markers were 1.3 cM and 4.7 cM, respectively. Phenotypic variance explained by this QTL was 63%, and the *O. glaberrima*-derived allele contributed a decreasing effect on GPP.

### Results

#### Phenotypic characterization and validation of qgnp7(t)

The 9IL188 plants were dwarfed and had obviously small panicles (Fig. 1A, 1B) when compared with the 9311 parent line. Phenotypic evaluation of agronomic traits showed that the differences between 9IL188 and 9311 in plant height, panicle length, grains per panicle and tillers per plant were significant ($P < 0.01$) (Fig. 1C–1F), while there were no significant difference in seed setting rate and 1,000-grain weight (Fig. 1G, 1H).

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### Substitution mapping of qgnp7(t)

To further refine the position of qgnp7(t), a larger F3 population containing 2,258 plants derived from the F2

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**Table 1.** PCR-based molecular markers used in qgnp7(t) mapping

| Markers | Type  | Position (kb)* | Forward primer (5′-3′) | Reverse primer (5′-3′) |
|---------|-------|----------------|------------------------|------------------------|
| RM436   | SSR   | 2,523          | ATTCTTCAGTAAAGACGCGG   | CTTCGTTGACCTCCCCAAC    |
| RM427   | SSR   | 2,655          | TCACTAGCTTCGCGCACC     | TGATGAGATTTGGTGAGGG    |
| RM298   | SSR   | 2,745          | CGATGAGCTTGGATCGATCT   | CAGTCCAAATGTCACAC     |
| 7Sui21  | Indel | 2,763          | GAAGCGAGAAGAAAAACAC    | ATCGAGGGGAGTCAGTAC     |
| SM2     | Indel | 2,773          | AAAGAATAAAGCCTGGGCG    | AGCTCCAAGCTTCCCA       |
| 7Sui24  | Indel | 2,804          | ATTATAAGGCTGAGGCGCT    | TCTCGCGGTGGTGTAGG      |
| 7Sui25  | Indel | 2,855          | TTGAGAAACAGAAAGAGTGA   | ACCCGGCTTTGAGCGAG      |
| RM82    | SSR   | 3,010          | TGGCTTCCTTGTCAATTGCC   | CGACTCTGGAGGAGTACG    |
| RM4986  | SSR   | 3,172          | GTCGTTAATGGATGATTGCTC  | CTGGTGACATGCTAAATTACA |

*Physical distance of markers in 9311 genome sequences.

**Table 2.** Primers used in qRT-PCR

| Name   | Forward primer (5′-3′) | Reverse primer (5′-3′) |
|--------|------------------------|------------------------|
| ACTIN1 | AGCAACTGGGATGATATGGA   | CAGGCCGATGAGGAACCA     |
| BG164  | TCGTACGGTGATTTGGCAATA  | ACCATAAGACGCTCCCAACCA  |
| BG165  | GATGTCCCTTCTGCCATGC    | CCACACCCCTTCTGGTTTT    |
| BG807  | CGGATGTGCGGCCGTCGA     | TCTGGCCTGGTGTAGG       |
| BG806  | ATAATGCTCCAAGACTCAATA  | TTTCAGGCCGATCTTGCAGAC  |
| BG166  | GCCAAGATTACAGTTCATTTGGC| TACTTAACTTCCATCTCA      |
| BG805  | TCTCCTGAGCTTCCAAGAGC   | CGAGGAGATCCAAGCTTGCAG  |
| BG804  | TCTACCCAAATCTCAGTCG    | AGGCCGATGATACGGGGTGC   |
| BG167  |CAAAGAAGGTGCGATGTTG    | GGCAATGACAGCTCTGAC     |
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There were 35 and 4 recombination events on the RM82 side and 20, 3 and 2, recombination events on the RM298 side (Fig. 4B). The mean phenotypic values of recombinant lines (L1–3) for PH, PL, and GPP were compared with that of recombinant lines (L4–5) at P < 0.001 level. Finally, according to their marker genotypes and phenotypic values, the position of \( q_{gnp7(t)} \) was mapped to a 41 kb interval defined by 7Sui21 and 7Sui24 (Fig. 4C).

Fig. 1. Phenotypic performance of 9311 and 9IL188 at Taicang experimental station. A. 9311 and 9IL188 plants at the mature stage. Scale bar = 10 cm, B. Panicle phenotypes of 9311 and 9IL188 at the mature stage, Scale bar = 3 cm. Comparison of plant height (C), grains per panicle (D), panicle length (E), tillers per plant (F), seed setting rate (G), and grain weight (H). Data presented are means with SD (n = 10 plants). ***, P = 0.001; NS, not significant at P = 0.05.

Fig. 2. The graphic genotype of the 9IL188 plant. The black and white chromosome segments were the \( O. \) glaberrima homozygote and 9311 homozygote, respectively. Only four chromosomes contained segments of donor parent, \( O. \) glaberrima. The other eight chromosomes were fixed with 9311.

heterozygous plants was constructed and genotyped with SSR markers RM298 and RM82. Sixty-two recombinants were identified and further genotyped with four additional Indel markers 7Sui21, SM2, 7Sui24, and 7Sui25 (Table 1). There were 35 and 4 recombination events on the RM82 side and 20, 3 and 2, recombination events on the RM298 side (Fig. 4B). The mean phenotypic values of recombinant lines (L1–3) for PH, PL, and GPP were compared with that of recombinant lines (L4–5) at P < 0.001 level. Finally, according to their marker genotypes and phenotypic values, the position of \( q_{gnp7(t)} \) was mapped to a 41 kb interval defined by 7Sui21 and 7Sui24 (Fig. 4C).
Characterization of qgnp7(t)

To validate the role of qgnp7(t) in rice development, we developed the following NILs from the F_4 generation: NIL-qgnp7, which carries the O. glaberrima-derived allele, and its matching line NIL-qGNP7, which carries the homologous segment from 9311. Correspondingly, the phenotypic evaluation of agronomic traits was investigated between the NILs at maturity. The largest differences detected between the two lines were for plant height and grains per panicle related traits (Table 4).

NIL-qgnp7 had a dwarf phenotype. The six internodes (from top to bottom) in NIL-qgnp7 were reduced by about 23.4, 30.6, 16.0, 26.2, 29.8, and 17.4% respectively, compared with NIL-qGNP7 (Table 4). The elongation mode of each internode in NIL-qgnp7 was similar to that in NIL-qGNP7 and the internodes were just proportionally shortened (Fig. 6). According to the six modes of internode elongation for rice dwarf mutants (Takeda 1977), we classified the mode of internode elongation in qgnp7(t) genotypes

Candidate genes in the qgnp7(t) location interval

Based on the published sequence annotation for Oryza sativa indica and Oryza glaberrima (http://www.gramene.org/), eight predicted genes were identified in the qgnp7(t) location interval. Of these, one gene is a Glycolate oxidase gene (GLO5) and the others are still of unknown function. We comparatively aligned the coding region of the common genes between 9311 and O. glaberrima. Compared with the alleles in 9311, all the genes in O. glaberrima showed differences except for BGIOSGA025165 (ORGLA07G0029000). Potential genes and alignment results are listed in Table 3.

We also checked the expression levels of these genes in the young panicles of two parents at booting stage. Compared to the 9311 plants, five genes were down-regulated and two gene was up-regulated in the 9IL188 plants (Fig. 5). Besides, the expression of BGIOSGA025166 was not detected in both parents (Fig. 5).

Table 3. Information and comparative analysis of candidate genes in the qgnp7(t) location interval

| 9311 Gene ID | Gene annotation | O. glaberrima gene ID | Comparison between 9311 gene and O. glaberrima gene | ID%a |
|-------------|-----------------|-----------------------|-----------------------------------------------------|------|
| BGIOSGA025164 | protein coding  | ORGLA07G0028900       | 6 SNP                                               | 99   |
| BGIOSGA025165 | Peroxisomal (S)-2-hydroxy-acid oxidase, GLO5 | ORGLA07G0029000 | same                                               | 100  |
| BGIOSGA024807 | protein coding  | ORGLA07G0029100       | 1 SNP                                               | 99   |
| BGIOSGA024806 | protein coding  | No annotation          | 19 SNP and 24 deletion                               | 96   |
| BGIOSGA025166 | protein coding  | No annotation          | 9 SNP and 2 insertion                               | 53   |
| BGIOSGA024805 | protein coding  | ORGLA07G0029200       | 3 SNP                                               | 99   |
| BGIOSGA024804 | protein coding  | ORGLA07G0029300       | 5 SNP                                               | 99   |
| BGIOSGA025167 | protein coding  | ORGLA07G0029400       | 12 SNP                                              | 93   |

9311 was used as the reference.

a Amino acid sequence identity derived from the O. glaberrima and the 9311 qgnp7(t) candidate genes.
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structure, suggesting that qgnp7(t) plays an important role in the growth and development of rice.

| Table 4. Phenotypic performance of NIL-qGNP7 and NIL-qgnp7 |
| Agronomic traits                  | NIL-qGNP7          | NIL-qgnp7          |
|-----------------------------------|--------------------|--------------------|
| Plant height (cm)                 | 99.3 ± 1.75a       | 77.4 ± 1.51b       |
| First internode from top (cm)     | 29.9 ± 0.90a       | 22.9 ± 0.64b       |
| Second internode from top (cm)    | 18.5 ± 0.64a       | 12.8 ± 0.52b       |
| Third internode from top (cm)     | 14.8 ± 0.42a       | 12.5 ± 0.37b       |
| Fourth internode from top (cm)    | 8.0 ± 0.52a        | 5.9 ± 0.35b        |
| Fifth internode from top (cm)     | 3.6 ± 0.35b        | 2.5 ± 0.34b        |
| Sixth internode from top (cm)     | 1.8 ± 0.20a        | 1.5 ± 0.12a        |
| Panicle length (cm)               | 25.0 ± 0.70a       | 20.8 ± 1.01b       |
| Primary branches per panicle      | 15.3 ± 0.67a       | 11.0 ± 0.94b       |
| Secondary branches per panicle    | 64.4 ± 1.90a       | 24.6 ± 1.35b       |
| Grains per panicle                | 277.4 ± 12.63a     | 130.9 ± 9.01b      |
| Seed setting rate (%)             | 73.0 ± 0.56a       | 74.5 ± 0.60a       |
| 1,000-grain weight                | 27.9 ± 0.47a       | 27.5 ± 0.62a       |
| Tillers per plant                 | 5.4 ± 0.97a        | 6.8 ± 0.79b        |
| Grain yield per plant (g)         | 27.8 ± 0.90a       | 15.6 ± 1.01b       |

A different letter indicates a significant difference at P < 0.001 (the same as below).

Fig. 5. Relative expression of the qgnp7(t) candidate genes in two parents (9311 and 9IL188). Panicle materials were collected at Booting Stage. Data presented are means with SE (n = 3 plants). *, P = 0.05; ***, P = 0.001; NS, not significant at P = 0.05.

Fig. 6. The proportion of each internode to stem in NIL-qGNP7 and NIL-qgnp7. Data presented are means with SD (n = 10 plants). IN1–6 internode 1–6.

Fig. 7. The proportion of primary and secondary branches per panicle to total branches per panicle in NIL-qGNP7 and NIL-qgnp7. Data presented are means with SD (n = 10 plants). PB primary branches per panicle, SB secondary branches per panicle.

Discussion

Oryza sp. includes two cultivated species (O. sativa and O. glaberrima) that originated from O. rufipogon and O. barthii, respectively (Wu et al. 2017). O. sativa has a high yield and is cultivated worldwide while O. glaberrima has a low yield and is only cultivated in the south of the Sahara desert in West Africa. To gain insight into the genetic basis for complex traits in O. glaberrima such as yield, plant architecture, stress response, and domestication, we developed a set of 159 chromosomal segment substitution lines (CSSLs) by repetitive backcross and marker-assisted selection derived from a cross between African Oryza glaberrima and Asian Oryza 9311 with 9311 as the recurrent parent. In
plants may be caused by the lack of function of a single gene. We comparatively aligned the amino acid sequence encoded by the common genes between 9311 and O. glaberrima. This revealed that the mutations in O. glaberrima genes only caused amino acid changes or small partial deletions in the amino acid sequences except for BGIOSGA025166 (Table 3). BGIOSGA025166 has 2bp insertion mutation in O. glaberrima, which leads to premature termination of protein translation. The results of qRT-PCR showed that the expression level of seven genes varied in the qgnp7(t) candidate genes between two parents, while the expression of BGIOSGA025166 was not detected (Fig. 5). Among the seven genes, the expression level of BGIOSGA025165 (ORGLA07G0029000) was reduced by about five times in the 9IL188 plants, and that of BGIOSGA025167 (ORGLA07G0029400) was increased by about four times (Fig. 5). The changes of expression levels of these two genes were extremely significant. Further high-resolution mapping and genetic transformation experiments are required to confirm the candidate gene for qgnp7(t).

In previous reports, locations on chromosome 7 containing or overlapping the qgnp7(t) interval were reported to be an important target during Asian rice domestication selection for plant architecture and panicle structure (Li et al. 2006, Tian et al. 2006). Unlike Li et al. (2006) and Tian et al. (2006), our study was conducted based on a CSSL (9IL188) with introgression fragments derived from Africa’s Oryza glaberrima. Considering this, it is possible that the qgnp7(t) interval was not selected during the related domestication of African rice. In our study, the yield estimation of NILs (NIL-qgnp7 and NIL-qGNP7) showed that the yield of NIL-qGNP7 plants was significantly improved by selecting the 9311 allele (Table 4). Thus, the identification of qGNP7(t) would provide novel gene resource for O. glaberrima improvement programs. Over the past 30 years, New Rice for Africa (NERICA) varieties were developed from crosses between improved tropical japonica and Oryza glaberrima, which possess the fine characters of Asian cultivated rice and African cultivated rice, and have carved a special niche for itself among upland rice farmers in sub-Saharan Africa (Nassirou and He 2011, Saito et al. 2018). During this process, refined method of conventional breeding, specifically-developed anther culture and double-haploidization techniques were used to overcome sterility and to hasten the breeding process. According to our previous strategy for constructing chromosomal segment substitution lines, the qGNP7(t) interval could be directly introduced from 9311 into O. glaberrima through repetitive backcross and marker-assisted selection. Comparing with NERICA breeding programs, this can greatly improve breeding efficiency and shorten breeding cycle for related traits.

In summary, qgnp7(t) is a major QTL for grains per panicle. Understanding the molecular mechanisms underlying this locus would be valuable for identification and transference of favorable alleles in African rice improvement.
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programs. Studies of the genetic basis and function of qggnp7(t) are underway.

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