RESEARCH NOTE

Characterization and fine mapping of *NGP4c*(t), a novel gene controlling the number of grains per panicle in rice

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Introduction

Rice (*Oryza sativa* L.), the world's most important cereal crop, is the primary source of food and calories for about half of mankind (Khush 2005). However, owing to a gradual decrease in farmland area, the average annual increase in rice production has been decreasing (He *et al*. 2010). Nowadays, food shortage has become an urgent global problem that needs to be solved (Jeon *et al*. 2011). Breeding new high-yielding rice varieties should be one of the most promising ways to resolve the contradiction between food demands and supply (Zhu *et al*. 2010). Rice yield is a complex trait multiplicatively determined by three component traits: number of panicles, grain weight and number of grains per panicle (NGP) (Hua *et al.* 2002). Of these, NGP is shown to be highly correlated with yield and acts as a crucial component in determining rice yield (Luo *et al*. 2013). Therefore, dissection of its genetic basis would be of great value in breeding high-yielding rice varieties. During the last decade, although many QTLs/genes controlling the NGP trait have been mapped in rice (Tian *et al*. 2006; Ahmadi *et al*. 2008; Xing *et al*. 2008; Liu *et al*. 2009; Deshmukh *et al*. 2010; Zhang *et al*. 2013), only a few related genes have been cloned (Ashikari *et al.* 2005; Huang *et al.* 2009; Tabuchi *et al.* 2011), and the molecular mechanism of NGP trait formation is still far from clear.

Here, we have identified a distinct NGP mutant *ngp4c* in rice. Genetic analysis indicates that the *ngp4c* phenotype is controlled by a single recessive gene, tentatively named as *NGP4c*(t) and the *NGP4c*(t) gene was finally mapped to 81.7 kb region, where no gene involved in the NGP trait formation had been reported previously. Thus, the results from this study provide a basis for further cloning and functional analysis of the *NGP4c*(t) gene.

Materials and methods

Plant materials and mapping population

The *ngp4c* mutant was a spontaneous NGP trait mutant derived from Nipponbare (*O. sativa* L. ssp. *japonica*), in the experimental paddy field at Lingshui, Hainan, China, during the winter of 2011. F_1 and F_2 populations from a cross between *ngp4c* and Minghui 63 (*Oryza sativa* L. ssp. *indica*) were used to determine whether *ngp4c* was controlled by single nuclear gene. This F_2 population was also used to map the *NGP4c*(t) gene.

DNA extraction and PCR

Genomic DNA was extracted from leaf tissues using the CTAB method (Chen and Ronald 1999). The PCR mixture was mixed with 1 μL DNA (10 ng/μL), 0.4 μL primers (10 μmol/μL), 2 μL 10$\times$ buffer, 0.4 μL dNTP (10 mmol/L), 0.3 μL *Taq* (5 U/μL) and 15.9 μL double-distilled water. PCR reaction system was performed as follows: predenaturation at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 30 s; annealing at 52–55°C for 30 s; extension at 72°C for 40 s; with a final extension step at 72°C for 10 min. The PCR products were separated on 3.5% agarose gels, stained with ethidium bromide and photographed.

Molecular markers development

Simple sequence repeats (SSR) markers were obtained from Gramene (http://www.gramene.org/microsat/) based on the SSR linkage map constructed by McCouch *et al.* (2002).

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Insertion/deletion (InDel) markers were developed according to DNA polymorphism of the mapped region between the *japonica* rice cv Nipponbare and the *indica* rice cv 9311 (http://www.ncbi.nlm.nih.gov/BLAST/). All InDel markers were designed by Primer Premier 5.0 software (Premier Biosoft International, Palo Alto, USA). The InDel markers used in this study were as follows: InDel-230 (F: ACCCAAAGTTCGAAGTGTCC and R: CTTTGGATTAACGGGGGTGT); InDel-372 (F: GCACGTGTCAGACCACTGAT and R: ACAGTTTCGTGCCTGTTTCC); InDel-436 (F: CATGAGTGCCATTGTTGTCA and R: GCAGCATCAAAGATGAAGCA).

Results

Characterization of the ngp4c mutant

We investigated the major agronomic traits when the plants were matured completely. The number of grains per panicle (count the number of grain on per panicle) in the *ngp4c* mutant was significantly lower than that of the wild-type parents (figure 1, a&b). The plant height (the average of plant height from the base to the tip of last leaf), panicle length (measure the length from the neck node to the panicle tip) and seed setting rate (the number of filled spikelets divided by the number of total spikelets), reduced by 14.5, 7.6 and 47.5%, respectively. The 1000-grain weight (the ripened grains were dried at 40°C in a forced-air oven until constant weight for determining grain weight) increased by 7.7% while the tiller number (counting of the tillers per plant) was not affected remarkably when compared with their wild-type parent (table 1).

Genetic analysis of the ngp4c mutant

We developed an F_2 population between *ngp4c* and cultivar rice Minghui 63 and found that the F_1 generation exhibited wild-type phenotype. Among the 3187 F_2 individuals, 2355 were wild-type phenotype plants and 832 were NGP mutant phenotype plants. The separate ratio was 2355:832, and approximately equal to 2.83:1, which accorded with 3:1 ($\chi^2 = 1.0 < \chi^2_{0.05,1} = 3.84$). These results indicated that the NGP mutant phenotype of *ngp4c* was controlled by a single recessive gene, which was tentatively designed as *NGP4c*(t).

Table 1. Comparison of major agronomic traits between the *ngp4c* mutant and its wild-type parent.

Agronomic trait	Wild type (control)	*ngp4c*	Compared with control (%)
Plant height (cm)	99.5±0.9	85.1±1.2	−14.5*
Tiller number	12.5±0.3	12.3±0.4	−1.6
Panicle length (cm)	22.3±0.6	20.6±0.7	−7.6*
Seed setting rate (%)	82.9±1.7	43.5±1.2	−47.5*
1000-grain weight (g)	23.3±0.4	25.1±0.5	+7.7*

*Significantly different at $P = 0.05$.

Table 2. Total number of SSR markers, the number of polymorphic markers and the percentage of polymorphic markers on each chromosome.

Chromosome	Total number of SSR markers	Number of polymorphic markers	Percentage (%)
1	42	22	52.4
2	34	17	50.0
3	33	19	57.6
4	29	15	51.7
5	29	13	44.8
6	31	13	41.9
7	22	10	45.5
8	26	11	42.3
9	19	12	63.2
10	20	7	35.0
11	22	11	50.0
12	19	8	42.1
Total	326	158	48.5

Fine mapping of the NGP4c(t) gene

To map the *NGP4c*(t) gene, map-based cloning was carried out by using F_2 population obtained from the cross of *ngp4c* and Minghui 63. By screening 326 pairs of SSR markers scattered on rice chromosomes with proportional spacing, we found 158 pairs of markers exhibited polymorphisms between the two parents (table 2), and then these markers were used for analysing the linkage relationship with the *NGP4c*(t) gene. The mutant phenotype DNA pool and wild-type phenotype DNA pool were used for linkage relationship analysis. Finally, RM6314 and RM3866 on the long arm of chromosome 4 showed a linkage relationship with the *NGP4c*(t) gene. Thus, *NGP4c*(t) gene was broadly mapped between RM6314 and RM5424 with genetic distances 5.8 and 4.2 cM, respectively (figure 1c).

In the region between RM6314 and RM5424, InDel markers were developed for fine mapping. Based on 832 F_2 recessive individuals from *ngp4c*/Minghui 63, the *NGP4c*(t) gene was finally mapped between InDel-372 and InDel-436 with genetic distances of 0.5 and 0.2 cM, respectively, and the physical distance was about 81.7 kb (figure 1d). Part of electrophoresis results of InDel-436 in the F_2 population are shown in figure 1e. Further, 16 putative open reading frames (ORFs) were predicted in the 81.7 kb genomic region which are annotated by the Rice Genome Annotation Project (http://rice.plantbiology.msu.edu/) (table 3), and none of them has been reported to be involved in the NGP trait formation in rice previously.

Discussion

Rice yield is one of the most valuable traits in rice production. In the long run, development of high-yielding varieties is one of the most important goals in rice breeding (Hao and Lin 2010). NGP makes a major contribution to rice yield and

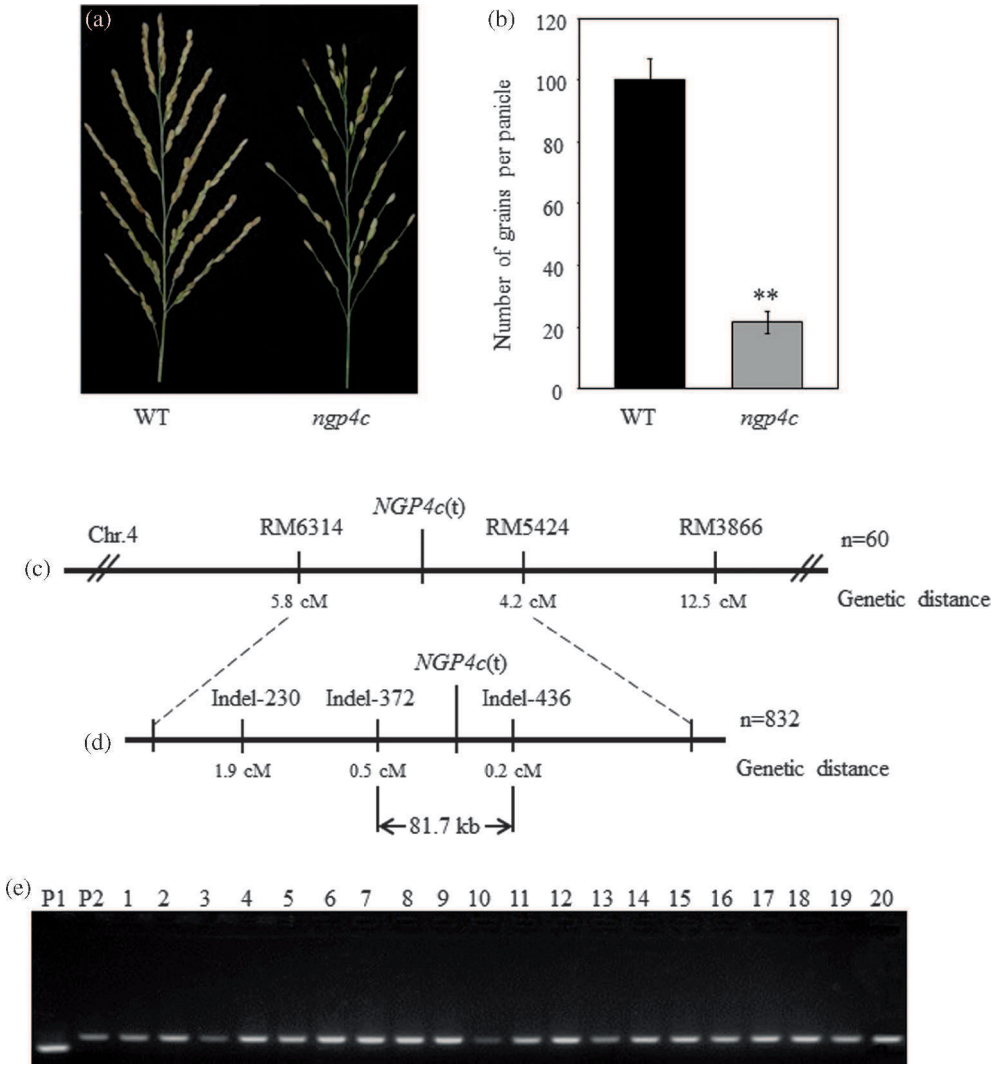

Figure 1. Comparison between the matured panicles of *ngp4c* mutant and the wild-type parent, and linkage mapping of *NGP4c*(t). (a) The number of grains per panicle of the *ngp4c* mutant was significantly decreased than that of the wild-type parent. (b) The statistics result of the number of grains per panicle, ** significantly different at $P = 0.01$. (c) Primary mapping of *NGP4c*(t). (d) Fine mapping of *NGP4c*(t). (e) Part of electrophoresis results of InDel-436 in the F_2 population derived from the cross of *ngp4c* and Minghui 63. P_1, Minghui 63; P_2, *ngp4c*; 1–20, NGP mutant plants in the F_2 population.

has been the focus in many genetic analyses (Kobayashi *et al*. 2001). Nowadays, the developments in genome mapping, sequencing and functional genomic research have provided powerful tools for investigating the genetic and molecular bases of the NGP trait, but the responsible loci and the related mechanism have not been fully understood at molecular level. Mutant analysis is a useful approach to illuminate the complex biological processes of NGP trait formation.

In this study, a spontaneous mutant *ngp4c* was identified in rice. The NGP of the *ngp4c* mutant was significantly decreased than that of the wild-type parent. Besides NGP trait, the plant height, panicle length and seed setting rate were also significantly decreased, while the 1000-grain weight was significantly increased in the *ngp4c* mutant, which indicated that *NGP4c*(t) should be a pleiotropic gene. Previous reports had shown that some genes had pleiotropic effects on many yield-related traits. For example, *Lk1* and *Lk2*, the long kernel and heavy grain weight genes, were identified from the mutants Nagayama 77402a (N179) and Nagayama 77402b (N182), respectively. *Lk1* mutant had long grain with low seed setting and reduced grain numbers, and *Lk2* mutant had long grain and accompanied with reduced panicle and grain numbers (Takamure *et al*. 1995). *GW2*, encoding a previously unknown RING-type protein with E3 ubiquitin ligase activity, which increased 1000-grain weight but decreased grains per main panicle (Song *et al*. 2007). *Ghd*7, encoding a CCT domain protein, also had major effects on an array of traits in rice, including number of grains per panicle, plant height and heading date (Xue *et al.* 2008). The above examples showed

Table 3. Candidate genes for *NGP4c*(t) in the 81.7 kb region on chromosome 4.

Gene ID	Putative function
LOC_Os04g32190	Expressed protein
LOC_Os04g32200	Expressed protein
LOC_Os04g32210	Expressed protein
LOC_Os04g32220	Expressed protein
LOC_Os04g32230	Hypothetical protein
LOC_Os04g32240	Retrotransposon, putative, centromere-specific, expressed
LOC_Os04g32250	Expressed protein
LOC_Os04g32260	Expressed protein
LOC_Os04g32270	Membrane-anchoured ubiquitin-fold protein, putative, expressed
LOC_Os04g32280	Hypothetical protein
LOC_Os04g32290	Expressed protein
LOC_Os04g32300	OsGrx_C9-glutaredoxin subgroup III, expressed
LOC_Os04g32310	Serine/threonine-protein kinase NAK, putative, expressed
LOC_Os04g32320	Glycerophosphoryl diester phosphodiesterase family protein, putative, expressed
LOC_Os04g32330	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial precursor, putative, expressed
LOC_Os04g32340	RNA-binding motif protein, putative, expressed

that it was not haphazard which genes had pleiotropic effects on related traits in rice.

To isolate the *NGP4c*(t) gene, a map-based cloning strategy was employed and the gene was finally mapped to an 81.7 kb interval between markers InDel-372 and InDel-436. Sixteen putative ORFs were predicted in the fine-mapped region. Among them, seven ORFs encoded expressed proteins; two ORFs encoded hypothetical proteins and one ORF encoded a retrotransposon. The products of other six ORFs were: a membrane-anchoured ubiquitin-fold protein, OsGrx_C9-glutaredoxin subgroup III, serine/threonine-protein kinase NAK, a glycerophosphoryl diester phosphodiesterase family protein, dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, a RNA-binding motif protein. None of these proteins have been reported to be involved in the NGP trait formation previously, suggesting that the *NGP4c*(t) gene should be a novel gene related to this important agronomic trait of rice.

In conclusion, we identified a spontaneous NGP mutant *ngp4c* in rice, characterized its mutant phenotype, and fine mapped its corresponding gene *NGP4c*(t). The results of this study lay a strong foundation for cloning of the *NGP4c*(t) gene and further elucidating the molecular mechanism of NGP trait formation in rice.

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