Fine mapping of the tiller inhibition gene *TIN5* in *Triticum urartu*

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Research Article

Keywords: TIN5, tiller number, fine mapping, *Triticum urartu*

Posted Date: January 20th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1260138/v1>

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Abstract

Grain yield in wheat (*Triticum aestivum* L.) is a polygenic trait representing many developmental processes and their interactions with environment. Among them, tillering capacity is an important agronomic trait for plant architecture and grain yield, but the genetic basis of tiller formation in wheat remains largely unknown. In this study, we identified a *tiller inhibition* mutant (*tin5*) from ethyl methane sulfonate (EMS) treated G1812 (*Triticum urartu* Thumanjan ex Gandilyan). A mapping population was constructed with *tin5*/G3146. Based on the sequence differences between G1812 and G3146, large InDels (≥ 5 bp) were selected and verified, and a skeleton physical map was constructed with genome-wide 168 polymorphic InDel markers. Genetic analysis revealed that the low-tiller phenotype was controlled by a single recessive locus, which we named *TIN5*. This locus was mapped to a 2.1-Mb region that contained 24 annotated genes in chromosome Tu7. Among these annotated genes, 16 genes expressed at the seedling stage, but only *TuG1812G0700004539* showed a non-synonymous polymorphism between *tin5* and WT. Our finding will facilitate its map-based cloning and pave the way for an in-depth analysis of the underlying genetic basis of tillering formation and regulation patterns.

Key Message

A tiller inhibition gene *TIN5* was delimited into an approximate 2.1 Mb genomic region on chromosome Tu7 that contains 24 annotated genes.

Introduction

Wheat (*Triticum aestivum* L.) is an important crop for global food security, providing about 20% of the calories and dietary proteins consumed by humans (FAOSTAT 2017). Wheat yield is usually dissected into three component traits, including productive tiller number per unit area (PTN, also referred to as spike number per unit area), number of seed per spike (SPS) and kernel weight (KW) (Ma et al. 2007), among which PTN is mainly affected by tiller number per unit area (TN) (Elhani et al. 2007). As each tiller has the potential to form a fertile inflorescence, the number of tiller is a critical determinant of grain yield (Sakuma et al. 2019). Additionally, tiller number per plant (TNP) is also a crucial trait for plant architecture (Naruoka et al. 2011). Despite its importance for grain yield, the molecular basis of tiller initiation is largely unknown in wheat due to tiller is a complex trait controlled by multiple genes and their interactions with environmental factors (Kebrom et al. 2012). Therefore, it is important to map and isolate genes responsible for tiller formation in wheat.

Over the past few decades, a number of genes controlling TNP have been isolated from the model plant species rice (*Oryza sativa*) and maize (*Zea mays*), including *Moc1* (Li et al. 2003), *IPA1/SPL14* (Jiao et al. 2010), *D53* (Jiang et al. 2013) and *MIT* (Liu et al. 2021). In wheat, the orthologous genes of *MOC1* and *IPA1/SPL14* mainly affect spike development, but not the tiller number (Cao et al. 2021; Zhang et al. 2015). *TEOSINTE BRANCHED1 (TB1)*, the most well-known tillering-related gene in maize, inhibits the growth of axillary organs under the high expression condition (Doebley et al. 1997). However, *TaTB1*

mainly increases the spikelet number per spike by promoting inflorescence branching (Dixon et al. 2018) and regulates plant height and stem internode length in wheat (Dixon et al. 2020). These findings suggested that the genetic control of tiller formation in wheat is somewhat different from that of rice and maize.

In wheat, at least six tiller inhibition genes have been reported, such as *tin1* (Richards 1988; Spielmeyer and Richards 2004), *tin2* (Peng et al. 1998), *tin3* (Kuraparthi et al. 2007), *ftin* (Zhang et al. 2013), *dmc* (An et al. 2019) and *TIN4* (Wang et al. 2021). One high-tillering dwarf mutant NAUH167 was characterized. Its TNP and plant height were controlled by a partial recessive gene mapped to the short arm of chromosome 2D flanked by markers *Xcfd11* and *Xgpw361* (Xu et al. 2017). Moreover, a number of QTLs affecting tiller number have been reported in wheat, including *QTn.mst-6B* (Naruoka et al. 2011), *QMTN.sicau-4D* (Hu et al. 2017), *QPtn.sau-4B* (Liu et al. 2018), *cqTN-2D.2* (Ren et al. 2018), *Qltn.sicau-2D* (Wang et al. 2019) and *Qetn-sau-1B.1* (Liu et al. 2020). Additionally, several genes were reported to associate with tiller formation in wheat. Overexpression of *tae-miR156* in common wheat (cv. Kenong199) led to an increased TNP (Liu et al. 2017). *PHYTOCHROME-INTERACTING FACTOR-LIKE (PIL)* family transcription factors were depressors of tillering in wheat. Over expression of *TaPIL1* reduces wheat TNP (Zhang et al. 2021). Down-expression of *TaPIN1s*, the auxin efflux carrier, increases the tiller number per plant of wheat (Yao et al. 2021). *TaD27* (the ortholog of rice *D27*), encoding an enzyme involved in the strigolactone biosynthesis in wheat, played a critical role in regulating wheat tiller numbers. *TaD27-RNAi* wheat plants had more tillers, and *TaD27-B-OE* wheat plants had fewer tillers. Unlike rice *D27*, plant height was not affected in the transgenic wheat plants suggesting that the divergent functions of *OsD27* and its wheat ortholog (Zhao et al. 2019).

Although a number of QTLs responsible for the tiller number have been reported, the genes underlying these loci have yet to be isolated and characterized. The difficulty of studying these genes is partially due to the complexity of wheat genome. The diploid wheat species *Triticum urartu* (AA, 2n = 14) is the wheat A-genome progenitor. Compared with common wheat, *T. urartu* has a smaller genome, making it an ideal model plant for studying complex agronomic traits. Moreover, the genome sequence of *T. urartu* acc. G1812 with 41,507 protein-coding gene models were released (Ling et al. 2018), which are useful for map-based cloning of genes from *T. urartu*.

In this study, we reported a *tiller inhibition* mutant (*tin5*) obtained from ethyl methane sulfonate (EMS) treated G1812, the accession used for the *T. urartu* genome assembly. The *tin5* mutant was defective in the outgrowth of axillary buds and harbored fewer tillers, compared to wild-type G1812. We finely mapped the low-tiller phenotype locus (herein named *TIN5*) with large F₂ segregating populations and predicted its candidate genes. This study provides a foundation for further cloning of *TIN5* and its uses in wheat breeding.

Materials And Methods

Plant Materials

The *tin5* mutant was obtained from 0.3% (v/v) EMS (Sigma-Aldrich) mutagenesis population (approximately 7,000 M₂ individuals) of *T. urartu* accession G1812 (PI 428198), a diploid wheat A genome progenitor with an available genome sequence (Ling et al., 2018). To map *TIN5*, an F₂ population derived from the cross of the *tin5* mutant and *T. urartu* accession G3146 (PI 538737) was developed. F_{2:3} families subsequently produced by self-pollination of recombinant individuals selected from the F₂ population were applied for fine mapping.

Growing conditions and phenotypic evaluations

The F₂ population and F_{2:3} families were evaluated at Zhaoxian county in Hebei province, China (37° 50' N, 114° 49' E) in wheat growing season 2016-2017 and 2017-2018, respectively. All the plant lines were sown as one-meter row plots with 11 plants, 10 cm between plants and 25 cm between rows. The seedlings were covered with plastic films in winter to avoid winterkill. Other field management was carried out in accordance with local wheat production practices.

The TNPs of 240 individual plants randomly selected from 2545 F₂ plants were counted manually at the grain filling stage in the field and their aboveground biomass (AGB) were measured at the maturity stage after air dried. For the rest F₂ plants and their selected F_{2:3} families, we divided the individuals into mutant-types and wild-types by visual inspection. Briefly, plant with 20 tillers or less and reduced plant height was characterized as low-tiller mutant, whereas the others were considered as wild type (high-tiller). The mutated phenotype of the key recombinants were confirmed by the corresponding F_{2:3} family segregation analyses.

Molecular marker development

Genomic DNA of all materials was extracted from leaves of young seedlings according to the CTAB method (Chatterjee et al. 2002). The accession G3146 was re-sequenced for a sequencing depth of 10x coverage, and the high-quality reads were aligned to G1812 reference genome (Ling et al. 2018). Genome-wide SNP and InDel between G3146 and G1812 were called using HaplotypeCaller module (McKenna et al. 2010). Based on the flanking sequence of InDels (sequence difference ≥5 bp), 440 InDel primers were developed. Additional simple sequence repeat (SSR) markers and SNP primers linked to *TIN5* were developed according to the G1812 genome sequence (Ling et al. 2018). The 10 µL PCR system contained 5 µL 2 × Taq PCR Starmix (GenStar, China), 3 µL H₂O, 1 µL of 80 ng/µL genomic DNA, and 0.5 µL of 10 µM each primer. The PCR reactions were carried out as described by Si et al. (2021). All the Primers used in the study are listed in Supplementary Table S1.

Genetic map construction and QTL detection

The genetic map was constructed with software JoinMap 4.0 using the Kosambi mapping function. QTL analysis was performed by inclusive composite interval mapping (ICIM) using the software IciMapping

4.1 (Zhao et al. 2020) with 1000 permutations at $P = 0.05$ in the F_2 population. A logarithm of odds (LOD) threshold of 2.5 was set to declare a significant QTL.

RNA isolation and quantitative reverse transcription PCR (qRT-PCR)

Total RNA was extracted from the tiller nodes at 4-leaf stage using TRIzol Reagent (Invitrogen, USA).

After removing genomic DNA contamination by DNase I (NEB, USA), SuperScript II (Invitrogen, USA) was utilized for first-strand cDNA synthesis according to the manufacturer's instructions. qRT-PCR experiments were performed using a total volume of 10 μ L with 4 μ L cDNA template, 1 μ L gene-specific primer (0.5 μ L of 10 μ M sense and 10 μ M antisense primers) and 5 μ L SYBR Green Master mix (TaKaRa, Japan) on LightCycler 480 Real-Time PCR System (Roche Diagnostics, Switzerland) according to the manufacturer's recommendations. The relative gene expression level was calculated using the $2^{-\Delta\Delta C_t}$ method with three biological replicates. The *ACTIN* gene was used as endogenous control (Zou et al. 2018). Primers for qRT-PCR are given in Table S1.

Results

Phenotype and genetic analysis of the reduced-tiller mutant

To investigate the molecular bases underlying wheat grain yield, we generated a population with approximately 7,000 M2 individuals in a *T. urartu* accession, G1812 (Ling et al. 2018), by ethyl methane sulfonate mutagenesis, and screened for mutants exhibiting altered important agricultural traits, including tiller number and plant height. One such mutant, *tiller inhibition* mutant (*tin5*), was selected for detailed study. Compared to G1812, young *tin5* plants were compromised in tillering ability in the field (Fig. 1a). The decreased tiller number per plant (TNP) of *tin5* was associated with the inhibition of tiller bud outgrowth rather than with fewer tiller buds (Fig. 1b-1d). At the heading stage, *tin5* exhibited reduced TNP and plant height compared with G1812 (Fig. 1e), and the average TNPs of G1812 and *tin5* were 44.87 and 17.53 (Fig. 1f), respectively.

A normal phenotype was observed for all F_1 plants derived from the cross between *tin5* and G3146, indicating that the tiller inhibition phenotype is a recessive trait. The F_1 plants were self-pollinated to generate F_2 mapping populations (total 2545 plants). Then, a genetic linkage analysis was performed by using 240 plants of the F_2 population, and the tiller habits of the corresponding individuals were recorded and verified in their $F_{2,3}$ families in the field at Zhaoxian, Hebei province. There were 188 normal plants and 52 reduced tiller plants in this F_2 population, which fitted a 3:1 Mendelian ratio ($188:52$; $\chi^2 = 1.25 < \chi^2_{0.05,1} = 3.84$). This result indicated that the reduced tiller phenotype is controlled by a single recessive gene.

Insertion and deletion (InDel) variation analysis between G1812 and G3146

In order to develop polymorphic markers between *tin5* and G3146, we re-sequenced G3146 and aligned high-quality reads to G1812 reference genome (Ling et al., 2018). Totally, 29,565 InDels between G3146 and G1812 were obtained by the HaplotypeCaller module. Among them, 1,747 InDels were larger than 5 bp (Table 1). Based on the flanking sequences of partial InDels (≥ 5 bp), 440 InDel markers were designed covering all seven chromosomes, and 168 (37.8%) markers were found to be polymorphic in 5% agarose gel visualization between *tin5* and G3146 (Table 1, Fig S1).

Table 1
InDels variations analysis between *T. urartu* accessions G1812 and G3146 at genome-wide level

Chromosome	No. total InDels		InDel markers development	
	No. InDels	No. InDels ≥ 5 bp	No. designed InDels	No. polymorphic InDels
Tu1	3,303	188	69	18
Tu2	5,820	354	78	26
Tu3	4,546	292	60	22
Tu4	3,296	172	58	25
Tu5	4,265	255	53	22
Tu6	3,014	172	60	27
Tu7	5,321	314	66	28
Total	29,565	1,747	444	168

Single-marker analysis and molecular mapping of TIN5

To map the *TIN5* locus, the same 240 F_2 plants of *tin5* $\times$ G3146 cross for genetic analysis were genotyped with 70 polymorphic InDel markers (ten markers on each chromosome). Among them, markers (*7T-33*, *7T-74* and *7T-81*) located on chromosome Tu7 were significantly ($P < 0.05$) associated with TNP and AGB (aboveground biomass) by single marker analysis performed on WinQTLCart version 2.5 (Table 2). The rest 18 polymorphic InDel markers on chromosome Tu7 were then used to genotype on the 240 F_2 plants. Subsequently, we chose 16 markers to construct the chromosome Tu7 genetic map of this F_2 population with a total genetic length of 33.3 cM (Fig. S2). With the genetic linkage map, QTL mapping for TNP and AGB was conducted with the additive model of inclusive composite interval mapping (ICIM). Finally, we found that *QTNP.ucas-7A* for TNP was coincident with *QAGB.ucas-7A* for AGB, indicating *TIN5* was mapped to the interval of 12.5 - 17.5 cM flanked by markers *7T-S117* and *7T-S187* (Fig. S2, Fig. 2a).

Table 2
Single marker analyses of TNP and AGB in an F₂ population

Trait	Marker	b0	b1	-2ln(L0/L1)	F(1,n-2)	pr(F)
AGB	7T-33	38.366	-8.102	6.687	6.784	0.0107*
AGB	7T-74	38.484	-7.23	5.543	5.588	0.0202*
AGB	7T-81	38.707	-9.166	8.325	8.521	0.0044**
TNP	7T-33	30.206	-5.548	5.951	6.013	0.0161*
TNP	7T-74	30.306	-5.246	5.56	5.606	0.0200*
TNP	7T-81	30.469	-6.656	8.366	8.565	0.0043**
TNP, tiller number per plant; AGB, aboveground biomass. *Indicates a significance level of $P \leq 0.05$; **Indicates a significance level of $P \leq 0.01$.						

Fine mapping of TIN5

The flank markers *7T-S117* and *7T-S187* were used to screen the remaining 2305 F₂ plants, and 46 recombinants were identified (Fig. 2b). To further narrow down the genetic region of the *TIN5* locus, we systemically genotyped 1,408 self-pollinating progenies (F₃) of the recombinants with the flanking markers (*7T-S117* and *7T-S187*), and with 7 newly developed polymorphic markers and phenotyped F₃ individuals by visual inspection. Taken together, *TIN5* was identified to co-segregate with the marker *7T-4188*, and was placed within a 2.1 Mb physical interval delimited by the markers *7T-S167* and *7T-S174* (Fig. 2c).

Mining of candidate genes

In the approximately 2.1 Mb genomic region defined by markers *7T-S167* and *7T-S174*, a total of 24 predicted genes (*TuG1812G0700004532* to *TuG1812G0700004555*) were found on the *Triticum urartu* v2.0 reference genome (Table S2). To identify the candidate genes for *TIN5*, we analyzed the orthologues of these 24 genes in rice. Among these genes, *TuG1812G0700004540* (*TuD27*) encoded beta-carotene isomerase D27 was the ortholog of rice *Dwarf 27* (*D27*) (Fig. S3). Rice *D27*, an iron-containing protein participating in the biosynthesis of strigolactones, play an important role in the regulation of tiller number in rice (Lin et al. 2009). Meanwhile, recent report has shown that *TaD27*-RNAi wheat plants had more tillers, and *TaD27*-B-OE wheat plants exhibited fewer tillers, suggesting that *TaD27* (the ortholog of *D27* in bread wheat) also plays a critical role in wheat tiller development (Zhao et al. 2019). Thus, *TuD27* may be the candidate gene of *TIN5*. To confirm that whether *TuD27* is responsible for the few tiller phenotype of *tin5*, we analyzed the promoter (1.6-kb), exon, intron and 3'-UTR region sequences of *TuD27*, and checked the expression levels of *TuD27* in the tillering nodes of *tin5* and WT. However, neither sequence nor expression difference were detected for *TuD27* between *tin5* and WT (Fig. S4a), indicating that *TuD27* is probably not the *TIN5* gene.

To narrow down the candidate genes of *TIN5*, we explored the expression patterns of other 23 genes beside *TuD27* within the *TIN5* mapping interval with the published G1812 expression data (Ling et al. 2018). Briefly, eight out of 23 genes were not expressed in any of three tissues (leaf, spike and root), and another 15 genes could be detected at least in one tissue (Table S3). We designed primers to amplify the exons, introns and about 1.2-kb of upstream promoter sequences of these 15 expressed candidate genes. We did not detect any changes in these genes between G1812 and *tin5*, except *TuG1812G0700004539*. The *tin5* mutant had a G → T base change causing single amino acid substitution from Aps to Tyr in *TuG1812G0700004539* (Fig. 3a). There was no obvious difference in the expression levels of *TuG1812G0700004539* between *tin5* and G1812 (Fig. 3b). *TuG1812G0700004539* encodes a putative 674 amino acid protein containing a typical pentatricopeptide repeat structure. *OGR1*, the homologue of *TuG1812G0700004539* in rice, encodes a pentatricopeptide repeat protein containing the DYW motif, is essential for RNA editing on five mitochondrial transcripts in rice. The *ogr1* mutant exhibited retarded growth, dwarfism and reduced tiller phenotype (Kim et al. 2009), which is similar to the morphological phenotypes of *tin5*. Thus, *TuG1812G0700004539* is most likely the candidate gene for *TIN5*.

Discussion

***T. urartu* with smaller genome facilitates the mining of genes controlling complex agronomic traits**

Although promising progress has been made in the assembly and annotation of the allohexaploid wheat genome, its large and complex genome is still a big restriction for functional genome of wheat (IWGSC 2018). In addition, most yield-related traits of wheat, especially tiller number, are quantitative traits affected by multiple gene loci and environmental conditions. So, it is difficult to isolate genes for the tillering traits (Kebrom et al. 2012). Till now, none of the underlying genes for tillering trait has been cloned in wheat through map-based cloning methodology (Wang et al. 2021). Therefore, researchers studied the homologues of the tillering genes cloned in model species, such as rice and maize, taking advantages of gene editing methodology. However, recent studies suggested that the homologous genes *TaMoc1* of *MOC1* in rice and *TaTB1* of *TB1* in maize mainly affect the spike development, but have little effects on tillering in wheat (Dixon et al. 2018; Zhang et al. 2015). These results indicated that the function of genes controlling tillering was differentiated between rice, maize and wheat. Therefore, it is of great significance to excavate the genes controlling tillering from diploid wheat relatives, such as *T. urartu*.

As the donor of wheat A subgenome, *T. urartu* is closely related to common wheat and has high genetic homology (Ling et al., 2018). Meanwhile, the smaller and simpler *T. urartu* genome compared to hexaploid wheat provides an opportunity to identify tiller mutants and clone corresponding genes in *T. urartu*. In the present study, we identified a *tin5* mutant with reduced tillering from the EMS mutagenesis library of *T. urartu* accession G1812, which genome was assembled (Ling et al., 2018).

An InDel map facilitates mapping of mutated genes

In order to improve the efficiency of marker development in this study, we called the InDel variations at the whole genome level between G1812 and G3146, and developed a physical map with 168 InDel markers covering seven chromosomes of *T. urartu* (Table 1). Yu et al. (2016) constructed a *T. urartu* genetic map containing 926 molecular markers. Among them, 584 DART markers need special equipment to reproduce. Since our InDel markers were developed from InDels larger than five base pairs, they can be easily distinguished by agarose gels (Fig. S1). Moreover, this set of markers has been used to preliminary map several *T. urartu* mutant genes under G1812 genetic background (data not shown) in our laboratory. So, these InDel markers are good supplement for published markers, especially for those using G1812 (PI 428198) as the material.

A novel gene or pathway regulating tillering in wheat

To date, a few tiller inhibition genes have been identified in wheat, such as *tin1*, *tin2*, *tin3*, *ftin*, *dmc* and *TIN4* (Wang et al. 2021). Meanwhile, several QTLs for tiller number were subsequently reported, such as *QTn.ipk-1B*, *Qetn-sau-1B.1*, *cqTN-2D.2*, *QPtn.sau-4B*, *QMTN.sicau-4D*, *QTn.ocs.5A.1* and *QTn.mst-6B* (Liu et al. 2020; Ren et al. 2018; Wang et al. 2021). However, no genes or QTLs for tiller number were isolated and cloned. Here, we mapped a tiller inhibition gene *TIN5* to a 2.1 Mb physical region on chromosome Tu7 of *T. urartu* genome. Among the 24 candidate genes, although *TuG1812G0700004540* is an ortholog of *Dwarf 27 (D27)* regulating rice tiller bud outgrowth (Fig. S3), there is no difference in its genomic sequences and at its expression level between the *tin5* and G1812 (Fig. S4a). Further, we didn't found significant differences at the expression levels of *TaD10* and *TaD17*, the downstream genes of *D27* in the strigolactone biosynthesis pathway in *Arabidopsis*, between *tin5* and G1812 (Fig. S4b, c). Therefore, *TuD17* was unlikely the gene responsible for reduced tiller phenotype in *tin5*.

Then, we sequenced 16 candidate genes expressed at least in young leaves, roots or spikes (Table S3). Compared to G1812, only *TuG1812G0700004539*, a putative protein containing pentatricopeptide repeat, had a SNP change (^G541^T) leading to an amino acid substitution (^D181^Y) in the *tin5* mutant (Fig. 3). In rice, pentatricopeptide repeat proteins mainly affect chloroplast development, and defects of these genes may lead to growth retardation, reduced plant height and tillering, and sterile phenotype (Kim et al. 2009; Toda et al. 2012). Compared with G1812, the leaf color of *tin5* did not change significantly (Fig. 1a). Based on these observations, most likely *TuG1812G0700004539* is the candidate gene of *TIN5*.

In summary, this study provided a set of low-density physical map of *T. urartu* constructed with agarose-resolvable InDel markers and finely mapped a new tiller inhibition gene *TIN5* on chromosome Tu7, and speculated the causal genes for *tin5*. *TuG1812G0700004539* has an SNP variation causing an amino acid change between the *tin5* mutant and WT. It is most likely the candidate gene for reduced tiller phenotype. The identification and mapping of *TIN5* will facilitate its uses in wheat breeding and the genetic dissection of molecular mechanism of wheat tillering.

Declarations

Funding

This research was jointly supported by grants from the National Natural Science Foundation of China (31971877) and the Strategic Priority Research Program of Chinese Academy of Sciences (grant no. XDA24010104-1).

Conflicts of interest/Competing interests

The authors declare that they have no conflicts of interest.

Availability of data and material

All data generated or analyzed during this study are included in the main text article and its supplementary files.

Code availability

Not applicable.

Authors' Contributions

SZ and YS developed the mapping populations; YS, QL, ST and JN carried out the experiments and analyzed the data; MC and XL assisted in phenotyping, genotyping and field work; QG and XS analyzed the data of re-sequencing; YS wrote the manuscript; H-Q L and SZ designed the project and revised the manuscript.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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Figures

Figure 1

Morphological features of the accession G1812 and the tiller-reduced mutant *tin5* of *T. urartu*.

(a) Seedling stage in the field; (b) Three-leaf stage, Bar, 2 cm.; (c) and (d) Magnified views of the boxed in (b); (e) Grain filling stage, Bar, 10 cm. (f) Histogram showing statistical analysis of the tiller numbers of G1812 and the *tin5* mutant at the grain filling stage. ($n = 15$). Double asterisks represent significant difference between G1812 and the *tin5* mutant in Student's *t*-test at $P < 0.01$.

Figure 2

Mapping of *TIN5* on chromosome Tu7.

(a) Primary mapping. (b) Fine physical mapping of *TIN5* using 2545 F2 individuals. Rec: number of recombinants. Numbers above marker indicate the number of recombinants. Red block indicates the candidate region of *TIN5*. (c) Graphical illustrations of the recombinant genotypes and phenotypes in the *TIN5* interval.

Figure 3

Structure of the candidate gene.

(**a**) Structure of *TuG1812G0700004539*. The black box shows the exon. Coding sequence (c.) and corresponding protein (p.). Nucleotide and amino acid sequences of G1812 and the *tin5* mutant are shown in red and black font, respectively. (**b**) Transcript levels of *TuG1812G0700004539* in G1812 and the *tin5* mutant at three-leaf stage. Error bars represent $\pm$ SD of the values of three biological repeats. Student's *t*-test ($**P < 0.05$) was used for statistical analysis.

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