Fine mapping of the tiller inhibition gene TIN5 in Triticum urartu

Yaoqi Si1 · Qiao Lu2 · Shuiquan Tian1,2 · Jianqing Niu1 · Man Cui1,2 · Xiaolin Liu1,2 · Qiang Gao3 · Xiaoli Shi1 · Hong-Qing Ling1,2 · Shusong Zheng1,2

Received: 14 January 2022 / Accepted: 23 May 2022 / Published online: 22 June 2022
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2022

Abstract
Key message A tiller inhibition gene TIN5 was delimited to an approximate 2.1 Mb region on chromosome Tu7 that contains 24 annotated genes.

Abstract Grain yield in wheat (Triticum aestivum L.) is a polygenic trait representing many developmental processes and their interactions with the environments. 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 5 (tin5) mutant from ethyl methane sulfonate 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 insertions and deletions (≥ 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 on chromosome Tu7. Among these annotated genes, only TuG1812G0700004539 showed a non-synonymous polymorphism between tin5 and the wild type. Our finding will facilitate its map-based cloning and pave the way for an in-depth analysis of the underlying genetic basis of tiller formation and regulation patterns.

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 seeds per spike (SPS), and seed weight (SW) (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 tillers 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 since tillering 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 Mocl (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, TaTBI1 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 (Kuraparthy et al. 2007), ftn (Zhang et al. 2013), dnc (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 quality trait loci (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), QIms.sicau-2D (Wang et al. 2019), and Qetm.sau-1B.1 (Liu et al. 2020). Additionally, several genes were reported to associate with tiller formation in wheat. The overexpression of tae-miR156 in common wheat (cv. Kenong199) led to an increased TNP (Liu et al. 2017). Conversely, PHYTOCHROME-INTERACTING FACTOR-LIKE (PIL) family transcription factors has been reported as depressors of tillering in wheat, overexpression of TaPIL1 reduces wheat TNP (Zhang et al. 2021). Similarly, down-regulation of TaPIN1s, the auxin efflux carrier, increased the tiller number per plant of wheat (Yao et al. 2021). TaD27, the ortholog of rice Dwarf27 (D27), encoding an enzyme involved in the strigolactone biosynthesis in wheat, played a critical role in regulating wheat tiller number. 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 the divergent functions of D27 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 not been isolated and characterized. The compensatory effects of homoeologous genes and the complexity of wheat genome are the big restriction for gene cloning studies of tiller. Plant material is the ideal model plant for studying complex agrometrics. Moreover, the genome sequence of T. urartu acc. G1812 with 41,507 protein-coding gene models was released (Ling et al. 2018), which facilitates the map-based cloning of genes from T. urartu.

In this study, we reported a reduced tiller mutant tin5 obtained from ethyl methane sulfonate (EMS)-treated accession G1812, from which genome was assembled. Compared with the wild type, tin5 was defective in the outgrowth of axillary buds, and had less tillers and aboveground biomass. 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 mutant tin5 was obtained from 0.3% (v/v) EMS (Sigma-Aldrich) mutagenesis population (approximately 7000 M2 individuals) of T. urartu accession G1812 (PI 428,198), from which the genome was assembled (Ling et al., 2018). To map TIN5, an F2 population derived from the cross of tin5 and T. urartu accession G3146 (PI 538,737) was developed. The F2:3 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 F2:3 families were evaluated at Zhaoxian County in Hebei Province, China (37° 50′ N, 114° 40′ E) in wheat growing season 2016–2017 and 2017–2018, respectively. All 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. The field management was followed by the local wheat production practices.

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

Molecular marker development

Genomic DNA was extracted from fresh leaves of young seedlings according to the CTAB method (Chatterjee et al. 2002). The accession G3146 was re-sequenced for a
sequencing depth of 10× genome coverage, and the high-quality reads were aligned to the G1812 reference genome (Ling et al. 2018). Genome-wide single nucleotide polymorphism (SNP) and InDel between G3146 and G1812 were called using the 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 dDH2O, 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 F2 population. A logarithm of the odds (LOD) threshold of 2.5 was set to declare a significant QTL.

**Sequence analysis and RT-PCR**

To identify the genetic lesion in tin5, we explored the expression patterns of candidate genes within the TIN5 mapping interval with the existing G1812 expression data (leaf, spike, and root) (Ling et al. 2018). Moreover, we analyzed the coding sequences, 3’-UTR region and approximately 1.6-kb promoter sequence upstream of candidate genes within the mapping interval from both G1812 and tin5 with the primers listed in Table S1.

The expression analysis was performed using tiller nodes from the greenhouse seedlings. The seeds of G1812 and tin5 were sown into pots (dimensions 25 × 25 × 30 cm) containing the mixture of vermiculite and peat (volume:volume, 1:1) supplied with the slow release fertilizer (Osmocote, 14–14–14) according to the manufacturer’s instructions. The plants were grown under the glasshouse conditions (16–18 °C; 16 h light: 8 h dark) for 4–5 weeks. The tiller nodes were sampled from tin5 and G1812 seedlings with three replicates when the fourth tiller nodes were fully developed. Total RNA was extracted from tiller nodes 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 μL with 4 μL cDNA template, 1 μL gene-specific primer (0.5 μL of each 10 μM sense and 10 μM antisense primers) and 5 μL 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 the endogenous control (Zou et al. 2018). Primers for RT-PCR are listed in Table S1.

**Results**

**Phenotype and genetic analysis of tin5**

To investigate the molecular basis underlying wheat grain yield, we generated a population with approximately 7000 M2 individuals in a T. urartu accession, G1812 (Ling et al. 2018), by the EMS mutagenesis, and screened for mutants exhibiting altered important agricultural traits, including tiller number and plant height. One such mutant, a tiller inhibition 5 (tin5) mutant was selected for detailed studies. Comparing to G1812, young tin5 plants were compromised in its 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–d). At the heading stage, tin5 exhibited reduced TNP and plant height compared with G1812 (Fig. 1e). The average TNPs of G1812 and tin5 were 44.87 and 17.53, respectively.

A normal phenotype was observed for all F1 plants derived from the cross between tin5 and G3146, indicating that the tiller inhibition phenotype is a recessive trait. The F1 plants were self-pollinated to generate F2 mapping populations (total 2545 plants). Then, a genetic linkage analysis was performed by using 240 plants of the F2 population, and the tiller habits of the corresponding individuals were recorded and verified in their F23 families in the field at Zhaoxian, Hebei Province. The TNP distribution of the 240 F2 plants was multimodal (Fig. 1f). There were 188 normal plants and 52 reduced tiller plants in this F2 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.

**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 for 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).

**Fig. 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. Distribution of tiller number per plant f and aboveground biomass g in the segregating F2 population (*n* = 240)

**Single-marker analysis and molecular mapping of TIN5**

Considering tillering is greatly affected by the environment, aboveground biomass (AGB) of the F2 population was also
selected as a proxy for tiller number in order to more accurately map the TIN5 locus in the study. Consistent with the tiller number trait, the AGB trait of the 240 F2 plants also presented continuous distribution (Fig. 1g). To map TIN5, the same 240 F2 plants of tin5/G3146 cross for genetic analysis were genotyped with 70 polymorphic InDel markers on chromosome Tu7 were then used to genotype the 240 F2 plants. Subsequently, we chose 16 markers to construct the chromosome Tu7 genetic map of this F2 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, which explained 18.28–26.59% and 26.65–33.80% of the phenotypic variations, respectively, indicating TIN5 was mapped to the interval of 12.5–17.5 cM flanked by markers 7 T-33 and 7 T-174 (Fig. 2c, Fig. 2a).

### Fine mapping of TIN5

The flanking markers 7 T-S117 and 7 T-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 (7 T-S117 and 7 T-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 7 T-4188 and was placed within a 2.1 Mb physical interval delimited by the markers 7 T-S167 and 7 T-S174 (Fig. 2c).

### Mining of candidate genes

In the approximately 2.1 Mb genomic region defined by markers 7 T-S167 and 7 T-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, plays an important role in the regulation of tiller number in rice (Lin et al. 2009). Meanwhile, a 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, TaD27 may be the candidate gene of TIN5. To confirm that whether TaD27 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 tiller nodes at the four-leaf stage of tin5 and G1812. However, neither sequence nor expression differences were detected for TuD27 between tin5 and G1812 (Fig. S3, S4a).

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

| Chromosome | No. of total InDels | InDel markers development | No. of InDels ≥ 5 bp |
|------------|---------------------|---------------------------|---------------------|
|            | No. of InDels       | No. of designed InDels    | No. of polymorphic InDels |
| Tu1        | 3303                | 188                       | 69                  | 18                  |
| Tu2        | 5820                | 354                       | 78                  | 26                  |
| Tu3        | 4546                | 292                       | 60                  | 22                  |
| Tu4        | 3296                | 172                       | 58                  | 25                  |
| Tu5        | 4265                | 255                       | 53                  | 22                  |
| Tu6        | 3014                | 172                       | 60                  | 27                  |
| Tu7        | 5321                | 314                       | 66                  | 28                  |
| Total      | 29,565              | 1,747                     | 444                 | 168                 |

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

| Trait    | Marker | b0      | b1      | 2 ln(L0/L1) | F(1, n-2) | pr(F)   |
|----------|--------|---------|---------|-------------|-----------|---------|
| AGB      | 7 T-33 | 38.366  | -8.102  | 6.687       | 6.784     | 0.0107* |
| AGB      | 7 T-74 | 38.484  | -7.23   | 5.543       | 5.588     | 0.0202* |
| AGB      | 7 T-81 | 38.707  | -9.166  | 8.325       | 8.521     | 0.0044**|
| TNP      | 7 T-33 | 30.206  | -5.548  | 5.951       | 6.013     | 0.0161* |
| TNP      | 7 T-74 | 30.306  | -5.246  | 5.66       | 5.606     | 0.0200* |
| TNP      | 7 T-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 ≤ 0.05; **Indicates a significance level of P ≤ 0.01
On the other hand, eight out of other 23 genes beside TuD27 were not expressed in all three tissues investigated (leaf, spike, and root), whereas the remained 15 genes showed expression at least in one tissue (Table S3). Notably, we did not detect any changes both in these 15 expressed genes and eight unexpressed 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). Furthermore, we analyzed the expression abundance of TuG1812G0700004539 and did not observed significant difference 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. From these results, we speculate that TuG1812G0700004539 may be 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, compensatory effects of homoeologous genes, and its large and complex genome are still the big restriction for gene cloning and functional studies in common 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 have been cloned in wheat through the 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*, a homolog of *MOC1* in rice, and *TaTB1*, a homolog 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. Here, we identified the tiller mutant tin5 from the EMS mutagenesis library of T. urartu accession G1812, finely mapped TIN5, and predicted the candidate genes. This research can be an example of studying genes for complex traits in T. urartu.

**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 (the wild type of tin5) 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 are DArT markers, which 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 428,198) as the material.

**The candidate genes for TIN5**

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, Qtn.sau-1B.1, cqTN-2D.2, QPttn.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 the chromosome Tu7 of T. urartu genome. Among the 24 candidate genes (Table S2), TuG1812G0700004540 (TuD27) is an ortholog of Dwarf 27 (D27) regulating rice tiller bud outgrowth (Fig. S3). Unexpectedly, no sequence difference was detected for TuD27 between tin5 and G1812 (Fig. S3). In rice, plastid-localized enzymes encoded by D27, D10, and D17 play crucial roles in catalyzing the formation of carlactone, which is further catalyzed by cytochrome P450 to generate strigolactones involved in tiller regulation (Zhang et al. 2014). Therefore, we further analyzed the relative expression levels of TuD27, TuD10 (TuG1812G030000321), and TuD17 (TuG1812G0200004646) (the orthologs of D10 and D17 in rice, respectively) in the tiller nodes at the four-leaf stage between tin5 and G1812. No significant expression differences were observed for TuD17, TuD10 and TuD17 between tin5 and G1812 (Fig. S4). From these results, it is still hard to rule out TuD27 as a candidate gene. For instance, a candidate gene Csi for tin1 in hexaploid wheat was associated only with the variation in the length of a microsatellite repeat in the 5‘-UTR. In the study, any SNPs at the locus would be difficult to detect via sequencing due to alignment errors, and the expression difference was only evident at some particular time-points during early stem elongation (Hyles et al. 2017). A time-course experiment
of all candidate genes might be helpful in the future work for determining the causal gene of low tillers of tin5.

To anchor the candidate genes, we then sequenced other 15 genes, which were expressed in seedling leaves, roots or spikes (Table S3). Compared to the wild type G1812, only TuG1812G0700004539, a putative protein containing pentatricopeptide repeat, had a SNP change (G541T) leading to an amino acid substitution (D181Y) in the mutant tin5 (Fig. 3a). In rice, pentatricopeptide repeat proteins mainly affect chloroplast development, and defects of these genes led to growth retardation, reduced plant height and tillering, sterile phenotype, and leaf color changes (Kim et al. 2009; Toda et al. 2012). In contrast, the leaf color of tin5 did not change significantly compared with G1812 (Fig. 1a, e). To explore other possible candidate genes, we sequenced these eight unexpressed genes and found that there was no SNP for these genes between tin5 and G1812. Based on these observations, TuG1812G0700004539 is a strong candidate gene of TIN5. Since the transgenic method is yet to be developed in T. urartu, future studies will include editing the orthologous genes of TuG1812G0700004539 in common wheat and elucidating the molecular mechanisms of tillering in wheat.

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 predicted its causal gene. TuG1812G0700004539 has an SNP variation causing an amino acid change between tin5 and G1812. It is a strong 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.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00122-022-04140-w.

Author contribution statement SZ and YS developed the mapping populations; YS, QL, ST, and JN carried out the experiments and analyzed the data; ST analyzed the candidate genes; 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 developed the mapping populations; YS, QL, ST, and JN carried out the experiments and analyzed the data of re-sequencing; YS wrote the manuscript; H-Q L and SZ.

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.

Declarations

Conflicts of interest The authors declare that they have no conflicts of interest.

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

References

An J, Niu H, Ni Y, Jiang Y, Zheng Y, He R, Li J, Jiao Z, Zhang J, Li H, Li Q, Niu J (2019) The miRNA-mRNA networks involving abnormal energy and hormone metabolisms restrict tillering in a wheat mutant dmc-. Int J Mol Sci 20:4586

Cao J, Liu KY, Song WJ, Zhang JN, Yao YY, Xin MM, Hu ZR, Peng HR, Ni ZF, Sun QX, Du JK (2021) Pleiotropic function of the SQUAMOSA PROMOTER-BINDING PROTEIN-LIKE gene TaSPL14 in wheat plant architecture. Planta 253:44

Chatterjee A, Moulik SP, Majhi R, Sanyal SK (2002) Studies on surfactant-biopolymer interaction. I. Microrolometric investigation on the interaction of cetyltrimethylammonium bromide (CTAB) and sodium dodecylsulfate (SDS) with gelatin (Gn), lysisom (Lz) and deoxyribonucleic acid (DNA). Biophys Chem 98:313–327

Dixon LE, Greenwood JR, Benvivega S, Zhang P, Cockram J, Mellers G, Ramm K, Cavanagh C, Swain SM, Boden SA (2018) TEOSINTE BRANCHED1 regulates inflorescence architecture and development in bread wheat (Triticum aestivum). Plant Cell 30:563–581

Dixon LE, Pasquariello M, Boden SA (2020) Teosinte branched1 regulates height and stem internode length in bread wheat. J Exp Bot 71:4742–4750

Doobley J, Stec A, Hubbard L (1997) The evolution of apical dominance in maize. Nature 386:485–488

Elhani S, Martins V, Chitterly B, Royo C, Moral LFGd (2007) Contribution of main stem and tillers to durum wheat (Triticum turgidum L. var. durum) grain yield and its components grown in Mediterranean environments. Field Crop Res 103:25–35

FAOSTAT (2017) FAOSTAT https://www.fao.org/faostat/en/#data. Food and Agriculture Organization (FAO) of the United Nations

Hu YS, Ren TH, Li Z, Tang YZ, Ren ZL, Yan BJ (2017) Molecular mapping and genetic analysis of a QTL controlling spike formation rate and tiller number in wheat. Gene 634:15–21

Hyles J, Vaurtin S, Pettinof F, MacMillan C, Stachurski Z, Breen J, Berges H, Wicker T, Spielmeyer W (2017) Repeat-length variation in a wheat cellulose synthase-like gene is associated with altered tiller number and stem cell wall composition. J Exp Bot 68:1519–1529

IWGSC (2018) Shifting the limits in wheat research and breeding using a fully annotated reference genome. Science 361:eart7191

Jiang L, Liu X, Xiong G, Liu H, Chen F, Wang L, Meng X, Liu G, Yu H, Yuan Y, Yi W, Zhao L, Ma H, He Y, Wu Z, Melcher K, Qian Q, Xu HE, Wang Y, Li J (2013) DWARF 53 acts as a repressor of strigolactone signalling in rice. Nature 504:401–405

Jiao Y, Wang Y, Xue D, Wang J, Yan M, Liu G, Dong G, Zeng D, Lu Z, Zhu X, Qian Q, Li J (2010) Regulation of OsSPL14 by OsmIR156 defines ideal plant architecture in rice. Nat Genet 42:541–544
Kebrom TH, Chandler PM, Swain SM, King RW, Richards RA, Spielmeyer W (2012) Inhibition of tiller bud outgrowth in the tin mutant of wheat is associated with precocious internode development. Plant Physiol 160:308–318

Kim SR, Yang JI, Moon S, Ryu CH, An K, Kim KM, Yim J, An G (2009) Rice OGR1 encodes a pentatricopeptide repeat-DYW protein and is essential for RNA editing in mitochondria. Plant J 59:738–749

Kuraparthy V, Shippa S, Dhaliwal HS (2007) Identification and mapping of a tiller inhibition gene (tin3) in wheat. Theor Appl Genet 114:285–294

Li X, Qian Q, Fu Z, Wang Y, Xiong G, Zeng D, Wang X, Liu X, Teng S, Hiroshi F, Yuan M, Luo D, Han B, Li J (2003) Control of tillering in rice. Nature 422:618–621

Lin H, Wang R, Qian Q, Yan M, Meng X, Fu Z, Yan C, Jiang B, Su Z, Li J, Wang Y (2009) DWFARF27, an iron-containing protein required for the biosynthesis of strigolactones, regulates rice tiller bud outgrowth. Plant Cell 21:1512–1525

Ling H-Q, Ma B, Shi X, Liu H, Dong L, Sun H, Cao Y, Gao Q, Zhang S, Li Y, Yu Y, Du H, Qi M, Li Y, Lu H, Yu H, Cui Y, Wang N, Chen C, Wu H, Zhao Y, Zhang J, Li Y, Zhou W, Zhang B, Hu W, van Eijk MJ, Tang J, Witsenboer HMA, Zhao S, Li Z, Zhang A, Wang D, Liang C (2018) Genome sequence of the progenitor of wheat A subgenome *Triticum urartu*. Nature 557:424–428

Liu J, Cheng X, Liu P, Sun J (2017) miR156-targeted SBP-Box transcription factors interact with DWARF53 to regulate *TEOSINTE BRANCHED1* and *BARREN STALK1* expression in bread wheat. Plant Physiol 174:1931–1948

Liu J, Luo W, Qin N, Ding P, Zhang H, Yang C, Mu Y, Tang H, Liu Y, Li W (2018) A 55K SNP array-based genetic map and its utilization in QTL mapping for productive tiller number in common wheat. Theor Appl Genet 131:2439–2450

Liu J, Tang H, Qu X, Liu H, Li C, Tu Y, Li S, Habib A, Mu Y, Dai S, Deng M, Jiang Q, Liu Y, Chen G, Wang J, Chen G, Li W, Jiang Y, Wei Y, Lan X, Zheng Y, Ma J (2020) A novel, major, and validated QTL for the effective tiller number located on chromosome arm 1BL in bread wheat. Plant Mol Biol 104:173–185

Liu L, Ren M, Peng P, Chun Y, Li L, Zhao J, Fang J, Peng L, Yan J, Chu J, Wang Y, Yuan S, Li X (2021) *MIT1*, encoding a 15-cis-β-carotene isomerase, regulates tiller number and stature in rice. J Genet Genomics 48:88–91

Ma Z, Zhao D, Zhang C, Zhang Z, Xue S, Lin F, Kong Z, Tian D, Luo Q (2007) Molecular genetic analysis of five spike-related traits in wheat using RIL and immortalized F2 populations. Mol Gen Genomics 277:31–42

McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella K, Altshuler D, Gabriel S, Daly M, DePristo MA (2010) The genome analysis toolkit: A map reduce framework for analyzing next-generation DNA sequencing data. Genome Res 20:1297–1303

Narukaya Y, Talbert LE, Lanning SP, Blake NK, Martin JM, Sherman JD (2011) Identification of quantitative trait loci for productive tiller number and its relationship to agronomic traits in spring wheat. Theor Appl Genet 123:1043–1053

Peng Z, Yen C, Yang J (1998) Genetic control of olio-culms in common wheat. Wheat Inf Serv 26:19–24

Ren T, Hu Y, Tang Y, Li C, Yan B, Ren Z, Tan F, Tang Z, Fu S, Li Z (2018) Utilization of a Wheat55K SNP array for mapping of major QTL for temporal expression of the tiller number. Front Plant Sci 9:333

Richards R (1988) A tiller inhibitor gene in wheat and its effect on plant growth. Austr J Agric Res 39:749–757

Sakuma S, Golan G, Guo Z, Ogawa T, Tagiri A, Sugimoto K, Bernhardt N, Brassac J, Mascher M, Hensel G, Ohnishi S, Jinno H, Yamashita Y, Ayalon I, Schnurbusch T, Komatsuda T (2019) Unleashing floret fertility in wheat through the mutation of a homeobox gene. Proc Natl Acad Sci 116:5182–5187

Si Y, Zheng S, Niu J, Tian S, Shi X, He Y, Li Y, Li Y, Ling H-Q (2021) Fine mapping of hybrid necrosis gene *Ne1* in common wheat (*Triticum aestivum* L.). Theor Appl Genet 134:2603–2611

Spielmeyer W, Richards R (2004) Comparative mapping of wheat chromosome 1AS which contains the tiller inhibition gene (*tin*) with rice chromosome 5S. Theor Appl Genet 109:1303–1310

Toda T, Fujii S, Nomuuchi K, Kazama T, Tortuya K (2012) Rice *MPR25* encodes a pentatricopeptide repeat protein and is essential for RNA editing of *nad5* transcripts in mitochondria. Plant J 72:450–460

Wang Z, Shi H, Yu S, Zhou W, Li J, Liu S, Deng M, Ma J, Wei Y, Zheng Y, Liu Y (2019) Comprehensive transcriptionomics, proteomics, and metabolomics analyses of the mechanisms regulating tiller production in low-tillering wheat. Theor Appl Genet 132:2181–2193

Wang Z, Wu F, Chen X, Zhou W, Shi H, Lin Y, Hou S, Yu S, Zhou H, Li C, Liu Y (2021) Fine mapping of the tiller inhibition gene *TN1* contributing to ideal plant architecture in common wheat. Theor Appl Genet 135:527–535

Xu T, Biao NF, Wen MX, Xiao J, Yuan CX, Cao AZ, Zhang SZ, Wang XE, Wang HY (2017) Characterization of a common wheat (*Triticum aestivum L.*) high-tillering dwarf mutant. Theor Appl Genet 130:483–494

Yao FQ, Li KH, Wang H, Song YN, Li ZQ, Li XG, Gao Q-X, Zhang XS, Bie XM (2020) Down-expression of *TaPIN1s* increases the tiller number and grain yield in wheat. BMC Plant Biol 21:443

Yu K, Liu D, Wu W, Yang W, Zhang A (2016) Development of an integrated linkage map of einkorn wheat and its application for QTL mapping and genome sequence anchoring. Theor Appl Genet 130:53–70

Zhang B, Liu X, Xu W, Chang J, Li A, Mao X, Zhang X, Jing R (2015) Novel function of a putative *MOC1* ortholog associated with spikelet number per spike in common wheat. Sci Rep 5:12211

Zhang JP, Wu J, Liu WH, Lu X, Yang XM, Gao AN, Li QX, Lu YQ, Li LH (2013) Genetic mapping of a fertile tiller inhibition gene, *ftin*, in wheat. Mol Breeding 31:441–449

Zhang L, He G, Li Y, Zhang Z, Liu T, Xie X, Kong X, Sun J (2021) PIL transcription factors directly interact with SPLs and repress tillering/branching in plants. New Phytol 233:1414–1425

Zhang Y, van Dijk ADJ, Scaffidi A, Flematti GR, Hofmann M, Charnikova T, Verstappen F, Hepworth J, van der Krol S, Leyser O, Smith SM, Zwanenburg B, Al-Babili S, Ruyter-Spira C, Bouwmeester HJ (2014) Rice cytochrome P450 MAX1 homologs catalyze distinct steps in strigolactone biosynthesis. Nat Chem Biol 10:1028–1033

Zhao B, Wu TT, Ma SS, Jiang DJ, Bie XM, Sui N, Zhang XS, Wang F (2019) *TaD27-B* gene controls the tiller number in hexaploid wheat. Plant Biotechnol J 18:513–525

Zhao DH, Yang L, Xu KJ, Cao SH, Yan J, He ZH, Xia XC, Song XY, Zhang Y (2020) Identification and validation of genetic loci for tiller angle in bread wheat. Theor Appl Genet 133:3037–3047

Zou S, Wang H, Li Y, Kong Z, Tang D (2018) The NB-LRR gene *Pnm60* confers powdery mildew resistance in wheat. New Phytol 218:298–309

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.