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ORIGINAL PAPER

Identification and mapping of a tiller inhibition gene *(tin3)* in wheat

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Abstract Tillering is one of the most important agronomic traits in cereal crops because tiller number per plant determines the number of spikes or panicles per plant, a key component of grain yield and/or biomass. In order to characterize the underlying genetic variation for tillering, we have isolated mutants that are compromised in tillering ability using ethyl methanesulphonate (EMS)-based mutagenesis in diploid wheat (*Triticum monococcum* subsp. *monococcum*). The tillering mutant, *tiller inhibition (tin3)* produces only one main culm compared to the wild type with many tillers. The monoculm phenotype of *tin3* is due to a single recessive mutation. Genetic and molecular mapping in an F_2 population of diploid wheat located the *tin3* gene on the long arm of chromosome $3A^m$. One codominant RFLP marker *Xpsr1205* cosegregated with *tin3* in the F_2 population. Physical mapping of PSR1205 in a set of Chinese Spring deletion lines of group-3 chromosomes placed the *tin3* gene in the distal 10% of the long arm of chromosome 3A, which is a recombination-rich region in wheat. The implications of the mapping of *tin3* on chromosome arm $3A^mL$ are discussed with respect to putative orthologs of *tin3* in the 3L colinear regions across various cereal genomes and other tillering traits in grasses.

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Introduction

Changes in plant architecture have been central to the domestication of wild species. Tillering or the degree of branching determines shoot architecture. The architecture of the shoot system affects a plant's light harvesting potential, the synchrony of flowering and seed set and, ultimately, the reproductive success of a plant. Shoot branches and/or tillers arise from axillary shoot meristems and tiller buds, respectively, which form in the axils of leaves on the primary shoot axis. Isolation and characterization of mutants with altered patterns of shoot branching/tillering showed that they can affect the meristem initiation, as in *lateral suppressor* (*ls*) (Schumacher et al. 1999) and *blind* (*bl*) (Schmitz et al. 2002) of tomato, *pinhead* (Lynn et al. 1999) and *revoluta* (*rev*) (Lynn et al. 1999; Otsuga et al. 2001) of Arabidopsis or meristem out growth as in *more axillary growth* (*max*) (Stirnberg et al. 2002) and *decreased apical dominance* (*dad*) (Snowden et al. 2005) of Arabidopsis, and *ramosus* (*rms*) of pea (Sorefan et al. 2003); or both as in *supershoot/bushy* of Arabidopsis (Tantikanjana et al. 2001; 2004) and *teosinte branched1* (*tb1*) of maize (Doebley et al. 1997) and *monoculm1* (*moc1*) of rice (Li et al. 2003). Cloning and characterization of

various genes that affect lateral shoot branching or tillering indicated that many of them are regulatory elements such as MYB transcription factors (*blind*-tomato), GRASS family transcription factors (*lateral suppressor*-tomato, *lateral suppressor*-Arabidopsis and *monoculm1*-Rice) (Schumacher et al. 1999; Li et al. 2003), Homeodomain-Leucine-Zipper transcription factors (*revoluta*-Arabidopsis) (Lynn et al. 1999; Otsuga et al. 2001) and the TCP family of DNA-binding transcriptional regulators (*teosinte branched1*-maize) (Doebley et al. 1997).

In cereals, tillering is controlled mostly by a number of quantitative trait loci (QTL) (for review see Li and Gill 2004). In wheat, although a single gene responsible for tiller inhibition was mapped on chromosome arm 1AS (Richards 1988; Spielmeyer and Richards 2004), most of the underlying variation for tillering was found to be controlled by QTL. Kato et al. (2000) showed the presence of minor QTL for tillering associated with the vernalization gene (*VrnA*) on chromosome 5A of wheat. In spring wheat, QTL with significant effect on tiller number per plant were found to be located on 6AS and 1DS (Li et al. 2002). Using an intervarietal, chromosome 3A-specific, recombinant inbred line population of winter wheat, Shah et al. (1999) mapped a significant QTL ($R^2 = 19.4\%$) for tillering on chromosome arm 3AL. Buck-Sorlin (2002) reported a major QTL for tillering ($R^2 = 30.6\%$) in barley on chromosome arm 3HL. Furthermore, a mutant locus affecting tillering, *low number of tillers* (*lnt1*), was placed on 3HL in the morphological map of barley (Franckowiak 1996). In rice, a QTL displaying 46.16% heritability for tillering also was mapped on chromosome 1 (Wu et al. 1999), which is syntenic to group-3 chromosomes of *Triticeae* (Ahn et al. 1993; van Deynze et al. 1995; Gallego et al. 1998; Sorrells et al. 2003). Tiller per plant mutations and QTL affecting tillering were mapped in the other genomic regions besides group-3 chromosomes of *Triticeae* species. In barley, a recessive mutation *cul2* was mapped to the proximal region of chromosome arm 6HL (Franckowiak 1996; Babb and Muehlbauer 2003). The monoculm (*mc*) gene was mapped to the proximal region of chromosome arm 6RL of rye (Maleyshev et al. 2001). Identification and characterization of tillering mutants in barley (Franckowiak 1996; Franckowiak and Lundqvist 2002; Babb and Muehlbauer 2003), rye (Maleyshev et al. 2001) and rice (Li et al. 2003) indicated that tillering is simply inherited at the diploid level.

We produced an array of mutants in *Triticum monococcum* subsp. *monococcum* (Dhaliwal et al. 1987; our unpublished results) through chemical mutagenesis using ethyl methane sulphonate (EMS) and screened for mutants with altered tiller number in the M_2 generation. Among these, the *tiller inhibition* (*tin3*) mutant is of particular agronomic interest, because *tin3* plants almost completely lose their tillering ability producing only one main culm in contrast to the multiple tillers in wild-type plants. Precise mapping and isolation of the mutant loci controlling tillering is important to exploit the underlying genetic variation in tillering for cereal crop improvement. The objective of this study was to conduct chromosome, genetic and sub-genomic physical mapping of the *tin3* gene using molecular methodologies combined with unique wheat cytogenetic stocks.

Materials and methods

Plant material

Triticum monococcum subsp. *monococcum* is a domesticated diploid wheat, very closely related to *Triticum urartu*, the diploid A-genome donor of polyploid wheats. *T. monococcum* subsp. *aegilopoides* is a wild form of *Triticum monococcum* subsp. *monococcum.* Both accessions of *monococcum* subsp. *monococcum* (TA4342-96) and *T. monococcum* subsp. *aegilopoides* (TA4342-95) were originally obtained from the late Dr. B.L. Johnson of University of California-Riverside, California, USA. *T. monococcum* subsp. *monococcum* (TA4342-96) was mutagenized using ethyl methanesulphonate (EMS). Mutagenesis and mutant isolation is as reported previously (Dhaliwal et al. 1987). *T. monococcum* subsp. *aegilopoides* (TA4342-95) was used as a second polymorphic parent in the crosses involving the *tin3* mutant. Both lines are maintained by the Wheat Genetic and Genomic Resources Center, Kansas State University, Manhattan, USA.

Reciprocal crosses were made between the *tin3* mutant and TA 4342-95. From the *tin3* × TA4342-95 cross, an F_2 population of 89 individuals generated in spring 2003, was used for molecular mapping. A population of 398 F_2 plants was grown from the reciprocal cross (TA4342-95 × *tin3*), and only 100 F_2 plants were scored for F_2 phenotypic segregation studies. Individual plants were grown in the square pots (Hummert International Horticultural Supplies, Earth City, MO, USA) filled with Scotts Metro Mix 200 (Sun Gro Horticulture Canada CM Ltd). Plants were grown in a growth chamber with 16 h of light and 8 h of darkness and with diurnal temperatures of 13–18°C. Tiller data was recorded twice; once at first internode detection stage and second time at flag leaf

sheath extending stage (for growth stage description see Zadoks et al. 1974). F_2 plants with single culm were characterized as having the *tin3* phenotype, whereas plants with more than one tiller were considered as wild-type.

Ditelosomic lines (Sears and Sears 1978) of group-3 chromosomes, in which a specific chromosome arm pair is missing, were used to identify fragments hybridizing to specific chromosome arms. For deletion bin mapping of the *tin3* gene, eight lines of Chinese Spring (CS) with terminal chromosomal deletions in the long arms of group-3 chromosomes (Endo and Gill 1996) were used. Four deletion lines for 3AL and two each for 3BL and 3DL were used. Included in the four 3AL deletion lines were TA4526-L3 (3DS-3/3AL) and TA4536-L5 (5BS-5/3AL), which had much smaller terminal deletions in the long arm of chromosome 3A (Qi et al. 2003). Physical mapping localized *tin3* into the smallest consensus deletion bin to further leverage the mapping and genomic information from the NSF-funded wheat EST mapping resource (http://www.wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cg).

Molecular mapping of *tin3* gene

DNA isolation

Freshly collected leaf tissue was frozen and ground in liquid nitrogen. About 10–15 ml of extraction buffer (0.5 M NaCl, 0.1 M Tris–HCl, 50 mM EDTA, 0.84%(w/v) SDS) (pH 8.0) was added to the ground tissue and incubated at 65°C for 30–45 min. About 15 ml of chloroform: iso-amyl alcohol (24:1) was added and mixed vigorously and centrifuged at 8,000 × *g* for 15 min. DNA was precipitated by adding 1.5 volumes of ice cold 95% ethanol to the supernatant. The DNA pellet was washed and incubated in 70% ethanol, dried, dissolved in TE buffer and quantified either on a 0.9% agarose gel or by using a NanoDrop ND-1000 UV–VIS spectrophotometer (Agilent Technologies, Palo Alto, CA, USA).

Microsatellite analysis

To genetically map the *tin3* gene, a total of 295 microsatellite markers were selected on the basis of the map positions in common wheat (Röder et al. 1998); Gupta et al. 2002; Somers et al. 2004; Guyomarc'h et al. 2002a, b). PCR reactions were performed as described in the above reports. In general, polymerase chain reaction (PCR) amplifications were performed in 25 µl reactions with 2.5 µl 10 × magnesium-free PCR buffer, 1.5 µl magnesium chloride (25 mM), 2.5 µl dNTPs (2.5 mM each dNTP) and 1 µl each forward and reverse primer (100 pmol/µl) and 75 ng DNA in a PTC-200 thermal cycler (MJ Research, Waltham, MA, USA). SSR primer annealing temperatures varied from 50 to 60°C, depending on the primer. Amplified products separated in 2.5% high-resolution agarose (Gene Pure HiRes Agarose, ISC BioExpress, USA) gels made with 1 × TBE. After 3 h at 65V, amplified products were visualized by ethidium bromide staining. Some of the amplified products were resolved using 6.5% KB^{Plus} Gel Matrix supplied by LI-COR® in a LI-COR 4200 DNA sequencer (LI-COR Biosciences, Lincoln, NE, USA) following the manufacturer's instructions. PCR reactions for SSR markers analyzed through the LI-COR machine were the same as described above except that fluorescence-labeled forward M13 tail primers were used for PCR with a total reaction volume of 10 µl.

Restriction enzyme digestion and RFLP analysis

In order to find a closely linked marker and to use it to localize the *tin3* gene into a deletion bin, a total of 18 RFLP clones previously mapped on the long arms of group-3 chromosomes (http://www.wheat.pw.usda.gov/GG2/maps.shtml#wheat; Devos et al. 1993a; McGuire and Qualset 1996; Dubcovsky et al. 1996) were selected for a polymorphism survey and subsequent mapping studies. Eighty-eight F_2 individuals from the cross *tin3* × TA4342-95 were used to genetically map the *tin3* gene using RFLP probes.

About 20 µg of DNA was digested with 40 units of endonuclease (*Eco*RI, *Eco*RV, *Dra*I, *Hin*dIII, *Sca*I or *Xba*I) in the presence of an appropriate buffer, BSA (0.01 v/v) and RNAase (0.01 v/v) for a total volume of 35 µl. After 16 h incubation at 37°C, the reactions were stopped by adding 8 µl of gel-loading buffer (0.25% bromophenol blue, 30% glycerol in water). Digested product was then resolved in a 0.8% agarose gel made using 1 × TBE (Tris, boric acid and EDTA) on a horizontal gel apparatus for 18 h at 23V. DNA was transferred to Hybond N^+ membranes (Amersham Biosciences, GE Healthcare, USA) according to the standard protocols of Sambrook et al. (1989).

Prehybridization was done at 65°C for 14–16 h in a solution containing 5 × SSPE (0.15 M NaCl, 0.015 M $Na_2H_2PO_4$, 0.1 M EDTA), 10 × Denhardt's solution (0.2% Ficoll; 2 mg/ml BSA; 2 mg/ml polyvinylpyrrolidine (PVP), and 0.25 mg/ml salmon sperm DNA. The prehybridization solution was replaced with 15 ml of hybridization solution (5 × SSPE, 10 × Denhardt's solution, 1% sodium dodecyl sulphate (SDS), and 10% dextran sulphate, 0.5 mg/ml salmon sperm DNA.

Probes were labeled with (^{32}P) dCTP by the random hexamer method (Feinberg and Vogelstein 1983), purified through Sephadex G50 spin columns, denatured for 4 min, and added to the membranes. After 18–22 h of hybridization, membranes were given a brief wash with 2 × SSC and 30 min followed by a wash with 2 × SSPE (0.1% w/v SDS), and subsequently washed twice for 30 min with 0.5 × SSPE (0.1% w/v SDS). Hybridizations and all the washes were done at 65°C. Membranes then were exposed to X-ray film for 3–7 days.

Linkage analysis

The computer program Mapmaker (Lander et al. 1987) version 2.0 for Macintosh was used to calculate linkage distances using the Kosambi mapping function (Kosambi 1944) with an LOD threshold of 3.00.

Results

Morphology and inheritance of *tin3* gene

The *tin3* mutant plants almost completely lost their tillering ability, producing only one main culm, in contrast to the multiple tillers in wild-type plants (Fig. 1a). Mutant culms were much stronger and leaves were much stiffer and darker than the wild-type plants. Spikes were much larger (Fig. 1b) and occasionally produced sterile tertiary spikelets. Seed size was bigger in the *tin3* mutants than the wild-type plants. Awns were mostly crinkled in *tin3* mutant compared to the wild-type plants (Fig. 1b).

The F_1 hybrid between the mutant (*tin3*) and *T. monococcum* subsp. *aegilopoides* showed the wild-type phenotype with many tillers, which indicated that the *tin3* gene with monoculm phenotype is recessive to the wild type (with many tillers). The F_2 population segregated 67 wild type and 22 mutant phenotypes, which was a good fit for the monogenic segregation ratio of 3:1. Thus, the mutant phenotype was due to a single recessive gene that affects tillering in *T. monococcum*. Reciprocal crosses involving the mutant and TA4342-95 showed the wild-type phenotype in the F_1 and segregation of 78 wild-type and 22 recessive plants giving a monogenic segregation ratio of 3:1 in the F_2. This result indicated that the mutant phenotype was conferred by a single recessive nuclear gene.

Microsatellite analysis

A total of 295 genetically mapped, A-genome specific microsatellite markers were used to survey the polymorphism between two parents, *tin3* mutant (in *T. monococcum* subsp. monococcum acc. TA4342-96 background) and *T. monococcum* subsp. *aegilopoides* (accession TA4342-95). Out of 295 SSRs surveyed, 27 SSR markers were amplified using fluorescence-labeled forward primers and their PCR products were resolved in a LI-COR DNA sequencer. The remaining 268 SSRs were amplified using unlabelled primers and PCR products were separated in 2.5% high-resolution agarose gels. Of the total 295 SSRs surveyed for polymorphism, 75 SSRs showed null alleles in both parents, suggesting that the transferability of bread wheat SSRs to the A-genome diploids is about 74.6%. From the 220 SSRs that were amplified in one or both parents, 93

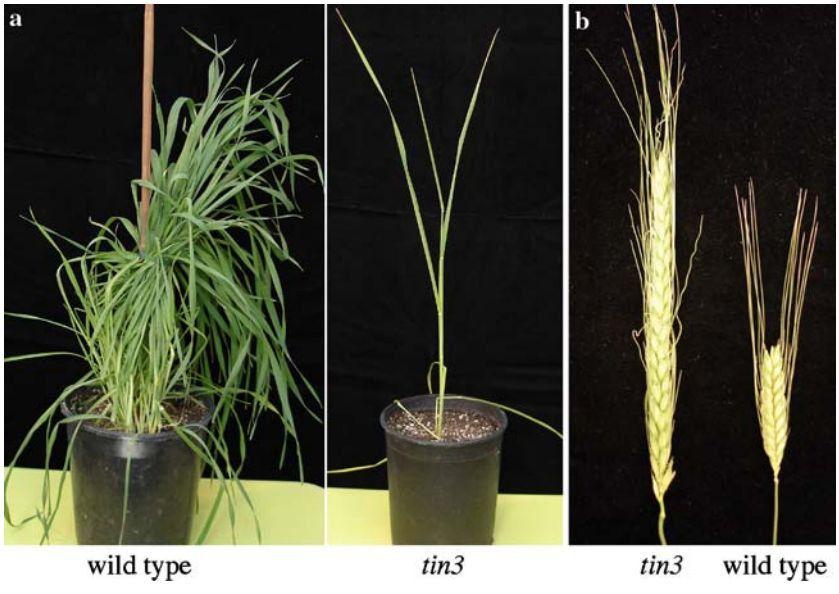

Fig. 1 Morphological features of *tin3* mutant and wild type *T. monococcum*. **a** Comparison of tillering abilities between wild-type and *tin3* mutant plants at maximum tillering stage. **b** Comparison of inflorescence or spike morphology between wild type and *tin3* mutant of *T. monococcum*

SSRs showed polymorphism (42.3%) between the two parents. Of the 93 polymorphic SSRs, 61 (65.6%) were co-dominant and 32 (34.4%) were dominant in nature. Of these dominant SSRs, 72% (23 SSRs) were dominant for *tin3* parent, whereas 28% (9 SSRs) were dominant for TA 4342-95 parent.

Molecular mapping of the *tin3* gene using SSR and RFLP markers

Genetic mapping

A total of 38 SSR (32 co-dominant and 6 dominant) markers were mapped in an F_2 population of 89 individuals from the cross *tin3* × TA4342-95. At an LOD score of 3.0, the Mapmaker's 'group' command identified the *tin3* locus grouping with two SSR markers *Xcfa2076* and *Xwmc169*. These two co-dominant microsatellite markers, which were genetically mapped on the long arm of chromosome 3A, showed close linkage (4.7 cM) with the *tin3* locus (Fig. 2). However, they were not physically mapped in the deletion bins of CS wheat (Sourdille et al. 2004). We used RFLP markers that could show orthologous alleles in the group-3 chromosomes to find a closely linked marker and to physically map the *tin3* gene using newly characterized deletion stocks specific to the group-3 chromosomes. Because SSR markers *Xcfa2076* and *Xwmc169* were mapped distally on the long arm of chromosome 3A (Gupta et al. 2002; Somers et al. 2004), we used 18 RFLP markers that were previously genetically mapped on the long arms of homoeologous group-3 chromosomes. DNA of the two parents was digested with six restriction enzymes (*Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Sca*I and *Xba*I). Sixteen probes (88.89%) detected polymorphism between the two parents with at least one enzyme, whereas the remaining two clones were monomorphic. Seven RFLP markers were mapped in the abovementioned F_2 population with only 88 individuals. All seven markers showed the same map order and relatively same marker distances as in previously reported group-3 chromosome maps (http://www.wheat.pw.usda.gov/GG2/maps.shtml#wheat, McGuire and Qualset 1996; Dubcovsky et al. 1996). One RFLP marker, *Xpsr1205*, co-segregated with the *tin3* locus in the above F_2 mapping population (Fig. 2). Probe PSR1205, which produced single band in both the parents *tin3* and *T. monococcum* subsp. *aegilopoides* (TA4342-95), also showed clear monogenic codominant marker segregation ratio of 1:2:1 ($p = 0.01$) in the F_2 population.

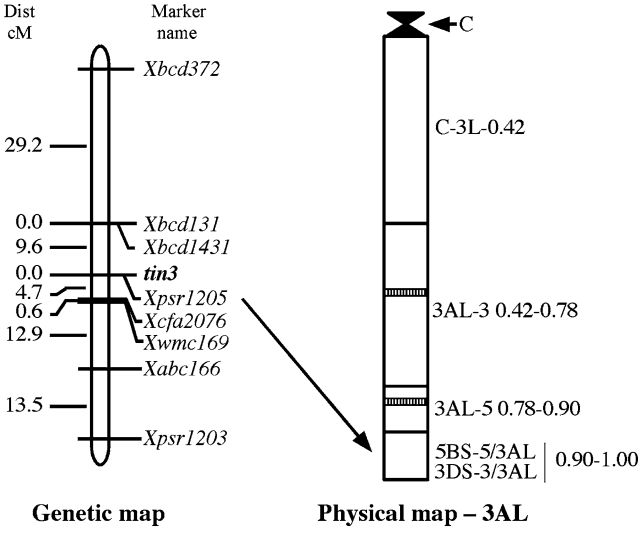

Fig. 2 Genetic mapping of *tin3* gene of *T. monococcum* (*left*) in reference to the physical (deletion) maps of chromosome arm 3AL (*right*). Orientation of the genetic map with respect to centromere is done by comparing the positions of the markers *Xbcd372*, *Xbcd131* and *Xpsr1205* reported in Dubcovsky et al. (1996) where the *top* of the map is towards the centromere. Mutant locus affecting tillering (*tin3*) is represented in *bold*. Each section of the 3AL physical map represents a bin delimited by deletion breakpoints expressed as fraction of arm length from the centromere. The fraction length (0.81–0.90) of the two new deletion lines 3DS-3/3AL and 5BS-5/3AL was tentatively based on Qi et al. (2003)

Physical mapping of the tin3 gene using deletion lines

In order to leverage the genomic information and tools developed in the wheat EST mapping project (http://www.wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cg), it was important to physically map the *tin3* gene using at least the core set of Chinese Spring deletion lines used in that project. Physical mapping of the *tin3* gene using the co-segregating marker *Xpsr1205* as a probe on a set of deletion lines revealed that *tin3* gene maps in the chromosome deletion bins 3AL-5 (FL 0.78–1.0), 3BL-7 (FL 0.63–1.00) and 3DL-3 (FL 0.81–1.00) of 3A, 3B and 3D chromosomes of wheat respectively (Fig. 3). In a consensus physical map of the group-3 chromosomes of wheat (Delaney et al. 1995) the *tin3* gene is positioned in the distal 20% of the long arms. We also used two new aneuploid lines, TA4526-L3 (3DS-3/3AL) and TA4536-L5 (5BS-5/3AL) of 3AL with smaller deletions for higher resolution physical mapping. The chromosome arm 3AL with same new terminal deletion is present in both lines, because identical sets of 3AL-specific EST fragments were missing in both lines (Qi et al. 2003). Both deletion lines TA4526-L3 (3DS-3/3AL) and TA4536-L5 (5BS-5/3AL) had much smaller deletions of less than 10% of the chromosome arm 3AL distal region (Qi et al. 2003). The

Xpsr1205 marker showed diagnostic polymorphism in these two new deletion lines also (Fig. 3), indicating that the *tin3* gene was actually located in the distal 10% of the long arm of chromosome 3A (Fig. 2).

Previously Shah et al. (1999) suggested that chromosome arm 3AL of hexaploid wheat also carries genetic factor(s) affecting tillering. The RFLP marker *Xbcd141*, which co-segregated with another RFLP marker *Xbcd372*, showed significant association with the tillering trait ($R^2 = 19.4\%$) in a recombinant inbred chromosome line population of chromosome 3A of wheat (Shah et al. 1999). We used BCD372 as a probe in our F_2 mapping population to explore the orthologous relationships between tillering QTL on chromosome arm 3AL of wheat (Shah et al. 1999) and *tin3* of *T. monococcum* subsp. *monococcum*. Mapping showed that *Xbcd372* is located about 38.8 cM proximal to *tin3* gene (Fig. 2).

Discussion

Tillering is an important component of grain yield in cereals. Identification and molecular characterization of genes involved in tillering is an essential prerequisite to elucidate the molecular mechanism of tillering for cereal crop improvement. The genetic and molecular mapping of the *tin3* mutant reported here provides a starting point for the molecular dissection of this trait in wheat.

Using the chemical mutagen EMS we isolated an array of mutants in *T. monococcum* subsp. *monococcum* including some with altered number of tillers. Among the tillering mutants, *tin3* is of particular agronomic interest because it produces only one main culm compared to 20–30 tillers produced by the wild type *T. monococcum* subsp. *monococcum*. The *tin3* mutant with a larger spike (Fig. 1), increased grain weight and darker leaves shows the agronomic importance of such loci and the potential need to understand the molecular mechanism of tillering for wheat improvement. Although *tin3* does not produce more than one tiller, the stronger culm, darker leaves and crinkled awns are few of the morphological similarities that this mutant shares with the barley mutant *low number of tillers1* (*lnt1*) mapped on 3HL (Franckowiak et al. 1996, Babb and Muehlbauer 2003), *lnt1*, however, differs from *tin3* by producing 2–3 tillers and also shows irregular rachis internode lengths.

Segregation in F_2 populations of reciprocal crosses between *tin3* × *T. monococcum* subsp. *aegilopoides* (TA4342-95) showed that *tin3* is a single recessive nuclear gene that is compromised in normal tillering ability. This result indicates that tillering is simply inherited in diploid wheat also, as has been reported in other diploid cereals such as barley (Franckowiak 1996; Franckowiak and Lundqvist 2002; Babb and Muehlbauer 2003), rye (Maleyshev et al. 2001) and rice (Li et al. 2003). The lack of many mutants that are compromised in tillering ability in hexaploid wheat might be due to the polyploid nature of wheat where the expression of functional homoeoalleles could be genetically compensating for the nonfunctional mutant locus. Identification and characterization of such simply inherited genes in diploids or putative diploid donors of polyploids will easily allow cloning and characterization of orthologous alleles for tillering not only in the polyploid wheat genome but in the *Triticeae* as a whole.

We employed SSR markers for chromosome and arm mapping of the *tin3* gene. Of the total 295 wheat

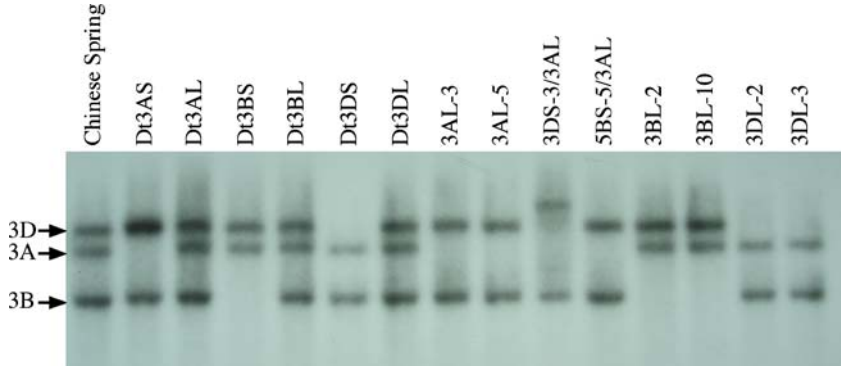

Fig. 3 Deletion bin based physical mapping of *tin3* gene by mapping co-segregating RFLP marker *Xpsr1205* in Chinese Spring group-3 aneuploid stocks. Autoradiograph shows the southern hybridization pattern of RFLP probe PSR1205 on Chinese Spring's ditelosomics (Dt) and deletion lines of group-3 chromosomes. The fraction length (0.81–0.90) of the two new deletion lines 3DS-3/3AL and 5BS-5/3AL was tentatively based on Qi et al. (2003)

A-genome specific SSRs surveyed, about 220 (74.58%) showed transferability to the A-genome diploids. A higher transferability (88%) of B-genome SSRs to the diploid species *Aegilops speltoides*, *Ae. longissima*, and *Ae. searsii*, representing the S genome was reported by Adonina et al. (2005). SSRs from *Ae. tauschii* to the D genome of wheat showed a still much higher level of transferability of 92% (Guyomarc'h et al. 2002a). Although our study involved only two A-genome diploid progenitors, the results indicated that among the polyploid A, B and D genome SSRs, the A-genome SSRs were comparatively less transferable to their corresponding diploid progenitors. Our present study also showed that out of 220 SSRs that amplified PCR products, 42.27% were polymorphic between the two parents used. The level of polymorphism of SSRs reported here was intermediate when compared to 33% reported between the parents of ITMI population (Gupta et al. 2002) and 60% polymorphism observed between Courtot and Chinese Spring (Guyomarc'h et al. 2002a).

Because the submicroscopic deletions of Chinese Spring are actually the result of terminal chromosome deletions (Endo and Gill 1996), any gene of interest can be mapped physically in chromosome bins by simply mapping either the closely linked proximal marker or the co-segregating marker(s) in the deletion lines. In the present study the nearest RFLP markers proximal to the *tin3* gene are *Xbcd131* and *Xbcd1431* which are 10.6 cM proximal to the *tin3* gene. However, one RFLP marker, *Xpsr1205*, showed co-segregation with the *tin3* locus in the same F_2 population (Fig. 2). This marker and *tin3* showed no segregation distortion in the F_2 population, and the marker *Xpsr1205* further shows all the three orthologous alleles in wheat (Fig. 3). Hence, we used PSR1205 as a probe to physically map the *tin3* gene.

Physical mapping of the *tin3* gene using the co-segregating marker *Xpsr1205* as a probe on a set of CS anueploid stocks including two new deletion lines (TA4526-L3 (3DS-3/3AL) and TA4536-L5 (5BS-5/3AL)) with terminal chromosome deletions revealed that *tin3* maps in the distal 10% of the long arms of group-3 chromosomes of wheat (Figs. 2 and 3). Deletion bin-based physical mapping of the *tin3* gene using CS deletion lines is important to know the genomic location of *tin3* gene on chromosome 3A with respect to recombination and gene space. Comparisons of the physical maps with recombination-based maps led to the discovery that gene density and recombination at the distal regions of the wheat chromosomes is very high (Werner et al. 1992; Gill et al. 1996; Akhunov et al. 2003) where gene density in such regions is comparable to that of rice (Feuillet and Keller 1999). The same trend of higher recombination in the distal 20% of the long arms of group-3 chromosomes was also demonstrated unequivocally by Delaney et al. (1995). Thus, physical mapping of *tin3* in such high recombination regions of the genome could allow us to undertake the map-based cloning of the *tin3* gene. Furthermore, deletion bin mapping of the *tin3* gene in a defined set of deletion lines used by NSF-EST mapping project (http://www.wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi; Qi et al. 2003) of wheat will allow us to access the deletion bin mapped ESTs as markers and comparative genomics tools such as macro and micro colinearity between rice and wheat (Sorrells et al. 2003). Because the group-3 chromosomes of wheat show a good level of colinearity with chromosome 1 of rice (Kurata et al. 1994; van Deynze et al. 1995; Sorrells et al. 2003, Liu and Anderson 2003) deletion bin mapped wheat ESTs could be leveraged either to fine map *tin3* and/or to explore the possible candidate genes of *tin3* from the syntenic BACs of the rice genome sequence.

So far, no orthologous mutant(s) defective in tillering ability has been reported on chromosome arm 3AL of hexaploid wheat. However, in a mapping study aimed at exploiting the intervarietal crop genetic variation, Shah et al. (1999) mapped a QTL determining tiller number on chromosome arm 3AL using recombinant inbred chromosome lines of 3A developed from a cross between Cheyenne and chromosome substitution line Cheyenne (Wichita 3A). The RFLP marker *Xbcd141*, which cosegregated with another marker *Xbcd372*, showed significant association with the tillering trait ($R^2 = 19.4\%$) in the above RIL population. The mapping of *Xbcd372* about 29 cM proximal to *tin3* gene in our mapping studies (Fig. 2) indicates that the QTL reported on 3AL (Shah et al. 1999) and *tin3* gene of *T. monococcum* subsp. *monococcum* may not be true orthologs. However, the converse can also not be ruled out because the QTL cover relatively larger regions of the chromosomes.

Identification and mapping of the *tin3* gene on chromosome arm 3AL of diploid wheat has significance in the light of the conserved synteny widely reported in the comparative analysis of grasses (Devos and Gale 2000; Sorrells et al. 2003). Gene order is well conserved between wheat, barley and rye (Devos et al. 1993a, c; Dubcovsky et al. 1996) where the genomes of distantly related cereals such as maize, rice, oat and rye can be divided into linkage blocks that have homology to corresponding segments of the wheat genome (Ahn et al. 1993; van Deynze et al. 1995). Conservation of effective gene orthologs in the *Triticeae* were well documented

between wheat and barley in the case of vernalization genes *Vrn1* and *Vrn2* (Dubcovsky et al. 1998) and the photoperiod gene *Ppd1* (Snape et al. 1996). With respect to tillering in grasses, gene orthologies have not been demonstrated unequivocally. In addition to the *tin3* mutant reported here, a mutant with intermediate tillering habit, *low number of tillers1* (*lnt1*) was mapped on 3HL of barley (Franckowiak 1996). It was further speculated that the QTL TILL-1 ($R^2 = 30.6\%$) mapped on the long arm of chromosome 3H (Buck-Sorlin 2002) could be associated or identical with the major gene *lnt1* of 3HL. Although, no orthologous RFLP markers were reported for *lnt1*, the morphological similarities and arm location suggest that *tin3* and *lnt1* may be orthologs. In rye, a mutant locus affecting tillering *monoculm* (*mc*) was mapped on the long arm of chromosome 6R (Malyshev et al. 2001). The fact that the *Xpsr1205* locus could be detected on chromosome arm 6RL having a reciprocally translocated 3L segment (Devos et al. 1993a, c) and the common norm of reduced genetic recombination in the proximal regions of *Triticeae* chromosomes (Werner et al. 1993; Gill et al. 1996; Akhunov et al. 2003; Lukaszewski 1992; Devos et al. 1993a, b; Lukaszewski et al. 2004) indicates that *monoculm* (*mc*) of rye might be a putative ortholog of *tin3* of *T. monococcum* subsp. *monococcum*. Because a recessive mutation *cul2* was also mapped to the proximal region of chromosome arm 6HL (Franckwiak 1996), it would be interesting to see the true ortholog of *mc* of rye using the RFLP markers associated with *tin3* reported in the present study and *cul2* of barley (Babb and Muehlbauer 2003). In foxtail millet (*Setaria italica*), a major QTL ($R^2 = 28.1\%$) for tiller number was mapped at a genetic position of 115 cM on chromosome V (Doust et al. 2005). Cross species comparative genomic analysis of the markers in that critical region of foxtail millet shows an extreme level of colinearity with long arm of chromosome 1 of rice (Devos et al. 1998). Furthermore, among the many reported QTL affecting tillering in rice, a major QTL was also mapped on the long arm of chromosome 1 (Wu et al. 1999) of rice. It is very well established that the wheat chromosome 3L is syntenous to the long arm of chromosome 1 of rice (van Deynze et al. 1995; Sorrells et al. 2003). The above reports and mapping of *tin3* on 3AL of *T. monococcum* subsp. *monococcum* indicate that, cereal chromosomes that are collinear/syntenic to the long arms of group-3 chromosomes of *Triticeae* carry one or more genetic factors affecting tillering. Although the orthologous relationships between the QTL and/or mutant loci affecting tillering are not fully established, the mapping of the *tin3* gene of wheat in the present study will enable definitive comparative mapping of the orthologous tillering genes across the grass species described above. Precise mapping and isolation of the mutant loci controlling tillering is important to exploit the underlying genetic variation for tillering for cereal crop improvement. Even more importantly, cloning those genes of agronomic importance will further elucidate the molecular and cellular pathways in which these gene products function.

The diploid nature of *T. monococcum* with a smaller genome size of 5,700 Mb compared to 16,000 Mb of bread wheat (Arumuganathan and Earle 1991; Bennet and Smith 1976), the existence of a very high level of polymorphism for DNA based markers, the availability of a large BAC library (Lijavetzky et al. 1999), the physical map location of the *tin3* gene in a recombination-rich region of wheat and the extensive conservation of synteny between homoeologous group-3 chromosomes of wheat and chromosome 1 of rice (Ahn et al. 1993; van Deynze et al. 1995; Sorrels et al. 2003) makes the *tin3* gene a potential candidate for isolating tillering gene(s) using map-based cloning.

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