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SHORT COMMUNICATION

Assessment of Tiller Inhibition *(tin)* Gene Molecular Marker for its Application in Marker-Assisted Breeding in Wheat

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Abstract The tiller inhibition (*tin*) gene is known to reduce the number of tillers in a wheat plant, also leads to the development of 'Gigas' characteristics such as large spikes, thick leaves and stems. The 80 advanced lines with variable number of tillers were screened with a SSR marker (*Xgwm136*) associated with *tin* gene. 90 % lines amplified according to the reported banding pattern for *tin* gene. A nearly perfect co-segregation of the marker and the number of tillers per square meter area was observed suggesting that the marker can be used in the MAS for the number of tillers in wheat.

Keywords Wheat · MAS · *Tin* gene · *Xgwm* 136

Wheat yield potential in India has been sustained by evolving plant architecture over the last 50 years. The development of semi dwarf wheats with reduced plant height and responsiveness to inputs led to green revolution in 1960's. The dwarfing genes of the semi dwarf wheats allowed more effective tillers per plant, increasing biomass which further led to a dramatic shift in yield potential.

Tiller development is one of the many yield components which determine wheat plant architecture. Tillers arising as the main stem are called primary tillers and those from primary tillers are called secondary tillers [1]. The number of tillers per plant thus depends on tiller appearance and tiller survival [2], [3], [4]. Spikes borne on each tiller differ in their morphological characters such as compactness hence leading to different plant types [5]. Considerable genetic variation for this trait can be found in the wheat gene pool. Atsmon and Jacobs [6] have reported wheat genotypes with uniculm possessing enlarged spike and leaf morphology. This uniculm morphology of these genotypes was later on found to be determined by a single recessive gene [2], mapped to chromosome 1AS of wheat. However, the genetic basis for tillering is not well elucidated. The low-tillering lines produced a greater harvest index, fewer sterile tillers and a larger grain size [2], [7]. These factors demonstrate the agronomic importance of *tin* gene in wheat.

The application of molecular markers for quantitative trait locus (QTL) analysis to dissect complex traits in cereals was reported by Hodges [8]. There have been reports for number of QTL for tillering ability in rice [9–12], barley [13–15] and rye [16–18] but only few studies have been carried out in wheat. Law [19] observed that tiller number in wheat is controlled by some factor on chromosome 7B. Similarly Shah et al. [20] mapped a QTL for tillering in wheat on chromosome 3*AL*. Spielmeyer and Richards [21] identified a microsatellite marker (*Xgwm136*) tightly linked with *tin* gene was mapped on chromosome 1AS. They have suggested the use of this marker for marker-assisted selection of tillering in wheat. Li et al. [22] suggested for mapping QTLs for tillering in wheat using immortal populations. Recently, a new fertile tiller inhibition gene (*ftin*) has been reported and mapped to wheat chromosome 1AS [23].

To cope up the ever increasing demand of wheat in country, which will be 109 million tons by the year 2020 [24], there is a need to enhance the average productivity to more than 4.5 tons/ha. One of the approaches for achieving quantum jump in productivity is to restructure the wheat plant architecture. A new plant type was developed by Singh et al. [25] by utilizing a local germplasm SFW (Sirsa

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farm wheat). These lines had variable tillers per plant along with high 1,000 grain weight, high grain number per spike, higher biomass and thick stem. The SFW line has 2–3 tillers per plant with robust stem and large spikes. This study was based around the genotypes developed from hybridization between, SFW line and two released wheat varieties having high tillers per square meter (Vaishali and Vidisha). The second generation derivatives are being used in this study to assess the suitability of molecular marker (*Xgwm136*) linked to *tin* gene for evaluation of tiller numbers.

Data was recorded in a set of 80 such lines for number of tiller per square meter area. Observation was recorded three times in on plot and data was averaged to report the data on this trait. SSR marker *Xgwm 136* identified for *tin* gene was used for evaluation of these advanced lines. PCR reaction was conducted with primer sequences and amplification conditions as published in Röder et al. [26] and Graingenes (http://wheat.pw.usda.gov/index.shtml). Amplified PCR products were separated by electrophoresis in 2.5 % agarose gels according to the product size (HiMedia) at 4 v/cm in 0.5X TBE buffer.

Variation was observed in original parents (SFW and Vidisha) and in the set of advanced lines used in this study for the number of tillers per square meter. The parental line SFW possessed 203 tillers per square meter whereas the other parent 'Vidisha' showed a high value of 409. The *Xgwm136* profile for these parents was in accordance to Spielmeyer and Richards [21]. The average tiller number for the lines was 346 ± 8.26 (S.E.) tillers/m^2. Number of tillers per square meter ranged from 200 to 512 (Table 1). Out of 80 lines, 39 lines had significantly higher value for

Table 1 Pedigree details, number of tillers per meter and the banding profile of *Xgwm136* in a set of advanced lines

S. no.	Name	Pedigree	No. of tillers/ m^2	*Xgwm* 136	S. no.	Name	Pedigree	No. of tillers/ m^2	*Xgwm* 136
1	Parent 1	SFW (local wheat landrace)	203	350	42	Line 40	RNB 4 × DL 1266-16	220	350
2	Parent 2	Vidisha	409	300	43	Line 41	RNB 2 × DL 784-3	440	300
3	Line 1	SFW × DL 975//KAUZ ATILLA	416	300	44	Line 42	RNB 4 × H 1328-20-7-1	256	350
4	Line 2	DL 1270-7-1 × PBW 343	392	300	45	Line 43	H 1337-25-4 × DL 788-2	396	350
5	Line 3	SFW × DL 975//DL 1251	224	350	46	Line 44	H 1337-25-4 × DL 788-2	360	350
6	Line 4	DL 1270-7-1 × UP 2425	388	300	47	Line 45	H 1337-25-4 × DL 788-2	364	350
7	Line 5	DL 1266-1 × WR 957	272	350	48	Line 46	DL 1266-2 × H 943//DL 1266-2	284	350
8	Line 6	H 1337-25-4 × PBW 373	308	350	49	Line 47	UP 2425 × H 1337-25-4	420	300
9	Line 7	DL 1266-1 × UP 2425	252	350	50	Line 48	DL 1266-2 × H 943//DL 1266-2	364	350
10	Line 8	DL 1266-1 × UP 2425	268	350	51	Line 49	SFW × DL 784-3	360	350
11	Line 9	DL 1266-1 × H 1329-36-5	264	300	52	Line 50	SFW × DL 784-3	340	350
12	Line 10	DL 1266-1 × H 1329-80-4	480	300	53	Line 51	DL 1266-2 × H 943//DL 1266-2	200	350
13	Line 11	DL 1266-1 × H 1329-80-4	512	300	54	Line 52	H 1337-25-6 × PBW 343//H 1337-25-6	348	350
14	Line 12	H 1337-25-4 × H 1329-36-5	396	300/ 350	55	Line 53	H 1270-7-1 × PBW 343	444	300
15	Line 13	H 1337-25-4 × Cyt 1129-5	280	350	56	Line 54	DL 1266-2 × H 1314-6-2-1	420	300
16	Line 14	H 1337-25-4 × Cyt 1129-5	340	300	57	Line 55	H 1270-7-1 × PBW343*3	452	300
17	Line 15	RNB 1 x H 1337-25-6	332	–	58	Line 56	DL 1266-1 × Cyt 1129	412	300
18	Line 16	H 1337-25-4 × DL 784-3	464	–	59	Line 57	DL 1266-2 × H 943//DL 1266-2	352	300
19	Line 17	H 1337-25-4 × PBW 343//H 1283-19-2-1	272	350	60	Line 58	DL 1266-2 × HD 2859	464	300
20	Line 18	H 1337-25-4 × PBW343//H 1283-19-9-2-1	308	350	61	Line 59	H 1337-25-4 × PBW 343	388	300/ 350
21	Line 19	RNB 3 × DL 1266-2	260	–	62	Line 60	CBW 23 × DL 542	472	300
22	Line 20	DL 1266-1 × 20th ESWYT-43	264	350	63	Line 61	DL 1266-2 × HD 2859	392	350
23	Line 21	H 1337-25-4 × DL 784-3	364	350	64	Line 62	H 1337-25-6 × PBW 343//H 1337-25-6	424	300
24	Line 22	DL 1266-5 × H 1329-23-8-6	400	300	65	Line 63	H 1337-25-6 × PBW343	460	300
25	Line 23	DL 1266-5 × LOK 45	288	350	66	Line 64	L 2 × H 1314-6-2-1	400	300
26	Line 24	DL 1266-5 × RAJ 3765	312	350	67	Line 65	H 1337-25-4 × DL 784-3	412	300
27	Line 25	H 1337-25-4 × PBW 343	352	350	68	Line 66	H 1337-25-4 × Cyt 1129-5	336	350
28	Line 26	DL 1266-16 × DL 153-2	232	350	69	Line 67	H 1337-25-6 × H 1329-36-5	364	350
29	Line 27	DL 1266-16 × DL 153-2	352	350	70	Line 68	H 1337-25-6 × H 1283-19-2-1	368	350

Table 1 continued

S. no.	Name	Pedigree	No. of tillers/ m^2	*Xgwm* 136	S. no.	Name	Pedigree	No. of tillers/ m^2	*Xgwm* 136
30	Line 28	H 1382-1-5 × H 1314-6-2-1	344	300	71	Line 69	H 1337-25-6 × PBW 343//H 1283-19-2-1	328	350
31	Line 29	DL 1266-16 × DL 153-2	248	350	72	Line 70	RNB 1 × DL 1266-5	336	350
32	Line 30	H 1337-25-6 × PBW 343	280	350	73	Line 71	RNB 4 × H 1328-20-7-1	372	350
33	Line 31	H1270-7-1 × PBW343	388	300	74	Line 72	UP 2425 × H 1337-25-4	396	300
34	Line 32	H 1337-25-6 × PBW 343//H 1283-19-9-2-1	252	350	75	Line 73	DL 1266-2 × H 1314-6-2-1	416	300/350
35	Line 33	H 1337-25-6 × PBW 343//H 1383-19-9-2-1	316	350	76	Line 74	RAJ 3765 × 34th IBW 256	444	–
36	Line 34	L1 × DL 1266-5	216	350	77	Line 75	DL 1266-1 × H 1329-33-4	388	300
37	Line 35	L1 × DL 1266-5	320	350	78	Line 76	H 1382-1-5 × DL 1266-2	216	350
38	Line 36	RNB 1 × H 1396-10-18	308	350	79	Line 77	DL 1266-5 × Inqulab 9*2/Konak	228	350
39	Line 37	RNB 3 × H 1314-6-2-1	380	350	80	Line 78	DL 1266-5 × Inqulab 9/#2 Tukuru	284	350
40	Line 38	RNB 3 × DL 1266-16	376	350	81	Line 79	H 1337-25-6 × PBW 343//H 1337-25-6	324	350
41	Line 39	RNB 3 × DL 1266-16	244	350	82	Line 80	DL 1266-1 × WR 957	348	–

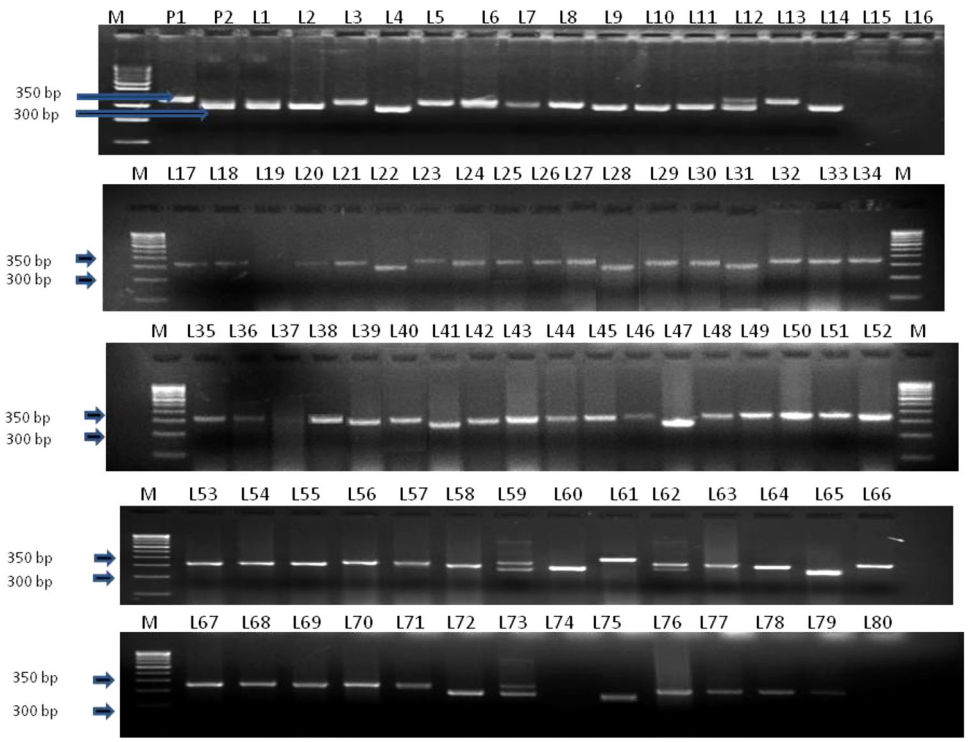

Fig. 1 Amplification pattern in a set of advanced lines for the microsatellite marker *Xgwm136* associated with *tin* gene in wheat

the number of tillers per square meter than the overall average value of 346 (C.D. at 5 % was 13.71). A total of 32 lines were found to have value for number of tillers per square meter significantly lower than the average value. Nine lines had the value of tillers per square meter at par with the overall average tiller number. Distinct banding pattern was observed in the lines for amplification with the *Xgwm136* (Fig. 1). Seventy two (90 %) lines showed either of the two bands whereas only eight lines showed no amplification or were heterozygous for the *tin* gene. 47 lines showed a band of 350 bp, 25 lines showed a band of 300 bp. Of the 25 lines showing the band for 300 bp, 21 had a significantly higher value for number of tillers per square meter. Three lines recorded values at par with the average number of tillers per square meter whereas only one genotype had a value lower than the average tillers.

Out of 47 lines which amplified for 350 bp, most had low values for number of tillers per square meter. 29 lines had number of tillers less than 332, which is significantly lower than the average value. Ten lines had values significantly higher than the average value whereas eight lines recorded tillers per square meter at par with the average value. Three lines amplified both the bands and also recorded a significantly higher value for number of tillers per square meter. Five lines showed no amplification.

This banding pattern in the lines obtained with *Xgwm136* is in accordance to the earlier report of Spielmeyer and Richards [21], suggesting the importance of this SSR in characterizing wheat lines. The microsatellite marker *Xgwm136* seems to be working correctly with this set of lines. A nearly perfect co-segregation of the marker and number of tillers per square meter length was observed. These results provide a better understanding of the association between *tin* gene and number of tillers per meter length. The molecular marker used in the study is able to differentiate between high and low tillering genotypes. This makes *Xgwm136* a probable candidate along with *Xcfa2153,* reported by Zhang et al. [23], for use in the marker assisted breeding for number of tillers in wheat. Detailed studies in wheat breeding programmes can further authenticate these results.

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