Agronomic evaluation of a tiller inhibition gene (tin) in wheat. II. Growth and partitioning of assimilate

B. L. Duggan¹, B.C., R. A. Richards¹, and A. F. van Herwaarden¹

¹CSIRO Plant Industry, GPO Box 1600, Canberra, ACT 2600, Australia.
²Research School of Biological Sciences, Australian National University, PO Box 475, Canberra, ACT 2601, Australia.
³Corresponding author; current address: CSIRO Plant Industry, Locked Bag 59, Narrabri, NSW 2390, Australia. Email: brian.duggan@csiro.au

Abstract. Wheats with reduced tillering have been proposed for areas regularly subject to a terminal drought. A wheat plant with a genetic disposition to produce fewer stems is now possible through the introgression of a gene that inhibits tillering (tin). This study was conducted to determine the effect of the tin gene on the dynamics of tillering, light interception, and dry-matter production and partitioning in several different cultivars of wheat. Commercial cultivars and their near-isogenic pairs differing in the presence of the tin gene were grown in well-watered tubes and also in the field in south-eastern Australia where terminal drought is common. Tiller number, light interception, leaf area index (LAI), biomass, and the partitioning of biomass were recorded at various intervals throughout the growing season. Water-soluble carbohydrate (WSC) levels in the stems of field-grown plants were also determined in some environments at anthesis and maturity. In tubes and in field environments, lines with the tin gene produced tillers at the same rate as their free tillering counterparts but ceased tillering sooner. Under conditions where the free tillering lines produced over 1000 shoots/m², lines containing the tin gene produced 600 shoots/m². However, by maturity, fertile spike numbers were 350 and 450/m² for lines with and without the tin gene, respectively. Despite the large difference in tillering, there were only small differences in LAI, light interception throughout the season, and biomass. There were small differences in the proportional allocation of biomass, and the tin lines partitioned more of their biomass towards spikes at anthesis and stored more WSC in stems. Dry weight distribution varied with genetic background, but in general the tin gene increased leaf area ratio and root to shoot ratio but decreased specific leaf area. It is concluded that the tin gene may be advantageous under terminal drought. This would come from the reduced light interception prior to anthesis and thereby potential for greater transpiration during grain filling as well as a greater capacity for stem carbohydrate storage and remobilisation. These factors are consistent with a greater harvest index and kernel weight associated with lines containing the tin gene.

Additional keywords: Triticum aestivum L., tillering, leaf area, root growth, stem carbohydrates.

Introduction

The concept of a higher yielding cereal crop consisting of reduced tillering plants was initially proposed by Donald (1968) on the basis that there would be little internal competition for assimilates between the developing spike and younger, possibly non-productive tillers. A uniculm wheat line that approached the Donald ideotype was described by Atsmon and Jacobs (1977) and tiller inhibition in this line was attributed to a single recessive gene, named tin (Richards 1988, Spielmeyer and Richards 2004). It was proposed that this gene might be advantageous not only under well-watered conditions originally considered by Donald but also in dry environments (Islam and Sedgely 1981, Richards 1988). Duggan et al. (2005) found, using near-isogenic lines in 4 different genetic backgrounds, that when sown at the same rate, on average grain yield was unaffected by the presence of the tin gene in terminal drought environments in south-eastern Australia. However, when sown at a higher sowing rate with spike numbers of the tin lines approaching that of their commercial near-isogenic pairs, there was a 2% yield advantage of tin lines and the highest yielding line contained the tin gene. The tin lines were not selected for yield in contrast to their near-isogenic parent lines. Yield components were altered with lines containing the tin gene. They produced fewer spikes at maturity, but compensated by producing more kernels/spike and kernels of greater weight than the
commercial lines. Harvest index (HI) was also greater for the

$t_n$ lines, whereas biomass was largely unaffected.

One of the proposed advantages of lines with the $t_n$

gene is that it could inhibit the growth of later formed
tillers that are largely unproductive. However, some authors
(Palta et al. 1994) have suggested that there were benefits
for fertile stems from the remobilisation of carbon and
nitrogen from unproductive tillers. Under low soil nitrogen
conditions, Palta and Fillery (1995) reported an increase in
the remobilisation of carbon and nitrogen when dry matter
was increased by N fertilisation. In contrast, when N supply is
in excess of what is required to optimise yield and
crops experience terminal drought (van Herwaarden et al.
1998a, 1998b), there is a greater potential retranslocation
of stem reserves to developing kernels when spike numbers are
low. This may be the result of a more open canopy
and less inter-plant competition (Gomez-MacPherson et al.
1998) associated with lower spike numbers allowing greater
penetration of light and therefore photosynthesis in the
lower leaves up to anthesis (van Herwaarden et al. 1998c).

Crops consisting of plants containing the $t_n$ gene may
therefore be able to accumulate greater levels of water-soluble
carbohydrates (WSC) in their stems as they typically produce
lower spike numbers than their freely tillering near-isogenic
pairs (Duggan et al. 2005). The thicker stems produced by
$t_n$ lines may also have a greater storage capacity for pre-
anthesis assimilated carbon. This could be of particular
importance in regions where terminal drought limits yield as
these crops may rely more on pre-anthesis stored assimilate
for grain filling than on current assimilation (Austin et al.
1977, 1980; Bidinger et al. 1977, 1980; Pheloung and Siddique
1991; van Herwaarden et al. 1998b).

It is proposed that lines containing the $t_n$ gene accumulate
biomass at the same rate as their freely tillering near-isogenic
pairs but partition it into fertile rather than non-productive
stems. The aims of the experiments reported here were to
investigate how the $t_n$ gene affects tiller development,
leaf area, and the partitioning of assimilate during the
early development of the wheat plant under controlled
conditions as well as the dry matter partitioning to the
remobilisation of WSC during grain filling.

Materials and methods

Tube experiment

Two seeds of the spring wheat cultivars Banks and Kite and their
near-isogenic lines containing the $t_n$ gene, Banks $+ t_n$ and Kite $+ t_n$,
respectively, each weighing between 40 and 45 mg, were sown
outdoors in Canberra, ACT, Australia (35°19′S, 149°00′E, 600 m alt.),
in cylindrical PVC tubes (150 mm high, 87 mm diam.) containing a
sandy-loam soil, on 18 August 1995, and thinned to 1 plant per tube
shortly after emergence. Plants were watered weekly with Hoagland’s
solution (Hoagland 1937) and harvested near the time when the ligule
of the 2nd, 3rd, 4th, and 5th main stem leaves had emerged (13,
41, 45, and 51 days after sowing, respectively). Average maximum
daily temperatures ranged from 16.0°C to 19.3°C, average minimum
temperature ranged from 1.8°C to 7.7°C, and daylength increased from
10.7 h to 12.5 h over the duration of the experiment. At each harvest,
roots were washed to remove the soil and the number of tillers was
determined. Plants were separated into roots, main stem leaves and
pseudo-stem, and tiller leaves and tiller pseudo-stems, then dried at 70°C
for 48 h before being weighed. Leaf area of each plant at each harvest was
determined using a Delta T area measurement system (Williamsburg,
VA, USA) fitted with a RCA tube camera. Specific leaf area (SLA) was
determined from the quotient of leaf area to mass of leaves plus stems.
There were 4 replicates of each genotype at each harvest.

Results

Near-isogenic pairs of both Banks and Kite commenced
tillering at the same stage of development, however, the
rate of tillering rapidly decreased in both Banks + tin and Kite + tin soon afterwards compared with their isogenic counterparts (Fig. 1). Tillering had ceased by the time leaf 3 appeared tillering in Banks + tin and by the time leaf 4 appeared in Kite + tin, whereas tillering continued until the final harvest (appearance of leaf 5) in Banks and Kite. Tiller number at the final harvest was lower for Banks + tin than for Banks (2 and 7 tillers/plant, respectively) and for Kite + tin than for Kite (3 and 8 tillers/plant, respectively).
Despite the large differences in tiller number/plant between the near-isogenic pairs, differences in leaf area and total biomass were small and usually not significant (Fig. 1). The only significant differences in leaf area between the near-isogenic pairs were at the final harvest for the Kite pair. There was no significant difference in total biomass between the pairs at the final harvest. However, biomass of the tin lines was greater at some intermediate harvests (Fig. 1). The root : shoot ratio of all lines declined with progressive harvests (Fig. 1), the only exception being Banks + tin where the root : shoot ratio was highest at the final harvest. The root : shoot ratio of Banks + tin was significantly greater than that of Banks in the final 2 harvests. No differences in root : shoot ratio were found in the Kite near-isogenic pair at any harvest.

The LAR and SLA of the tin lines were lower than of their normal tillering isogenic counterparts (Table 1), although this difference was only significant in the Banks pair of lines. There was no significant difference in the net assimilation rates for either near-isogenic pair between any harvest (data not shown).

Field experiments

Seedling emergence was the same for the cultivars and the tin lines at each site in any given sowing rate and, with the exception of C96N, averaged 85% of all seeds sown. At C96N, emergence was with only 84 and 89 plants/m² establishing for the cultivars and the tin lines, respectively, despite the seed being sown at a rate of 150 seeds/m². The presence of the tin gene resulted in a reduction in the maximum number of shoots/m² in all lines relative to their commercial parents at C96N and AP97 (Fig. 2a and b). Although the application of nitrogen at 50 kg/ha at C96N and at 160 kg/ha at AP97 increased total shoot number in the cultivars as well as in the tin lines, the increase in the tin lines did not approach the levels displayed by the cultivars. The reduction in tillering associated with the tin gene was due to the inhibition of later formed tillers. Final shoot number was reduced on average by 34% in the presence of the tin gene at C96N and by 39% at AP97 (Fig. 2a and b).

At C96N, the LAI of the tin lines differed significantly from the cultivars at anthesis (1.3 cf. 1.6) (Fig. 3). This was almost certainly a function of the poor establishment.
and the inability of the tin lines to produce tillers that would compensate for gaps in the canopy (Fig. 2a). At AP97, where establishment was good (average of 83% of seeds emerged), there were only small differences in LAI and light interception values for the tin lines and the cultivars. Table 2 shows light interception at AP97 at 3 growth stages. During the late vegetative stage (DC 16) there were no differences in light interception between cultivars with and without the tin gene. There was no difference in light interception of the tin lines was lower than of their commercial counterparts at the beginning of stem elongation (DC 30) and at anthesis (DC 65). There was no difference in light interception was 3% less for tin lines than for their free tillering counterparts.

The above-ground biomass of the cultivars and the tin lines did not differ significantly at any time between sowing and maturity (DC 92) at C95.1 or C95.2 (Fig. 4). At AP97, the cultivars had produced significantly more biomass at anthesis than the tin lines at either sowing density but by maturity the difference between the cultivars and the tin lines was no longer significant. Where there was poor establishment at C96 there was no difference in biomass between lines with and without the tin gene. There was little difference in the partitioning of above-ground biomass arising from the presence of the tin gene at anthesis in any experiment. The main difference was a higher proportion of ear weight relative to total weight of the tin lines. This was later reflected in a higher harvest index associated with tin lines (Duggan et al. 2005).

Stem WSC levels at anthesis were 9 and 5% higher for the tin lines than for the cultivars at M97 and at AP97, respectively, when compared at the same sowing rate, although the difference was not significant at AP97 (Table 4). By maturity, more WSC had been mobilised from the stems of the tin lines relative to the cultivars at M97. There was no significant difference in the amount of WSC mobilised for the tin lines at either sowing density but by maturity the difference between the cultivars and the tin lines was no longer significant. Where there was poor establishment at C96 there was no difference in biomass between lines with and without the tin gene. There was little difference in the partitioning of above-ground biomass arising from the presence of the tin gene at anthesis in any experiment. The main difference was a higher proportion of ear weight relative to total weight of the tin lines. This was later reflected in a higher harvest index associated with tin lines (Duggan et al. 2005).

### Table 2. Percentage light interception of commercial cultivars and lines containing the tin gene (at 2 sowing rates) at 3 rates of nitrogen (0, 80, and 160 kg/ha) at Ariah Park, 1997

<table>
<thead>
<tr>
<th>Nitrogen fertiliser</th>
<th>DC 16</th>
<th>DC 30</th>
<th>DC 65</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivars 0 30.0</td>
<td>71.7</td>
<td>72.2</td>
<td></td>
</tr>
<tr>
<td>Cultivars + tin (h.s.r.) 0 34.3</td>
<td>66.3</td>
<td>72.4</td>
<td></td>
</tr>
<tr>
<td>Cultivars 80 35.5</td>
<td>76.9</td>
<td>85.7</td>
<td></td>
</tr>
<tr>
<td>Cultivars + tin (h.s.r.) 80 39.8</td>
<td>72.8</td>
<td>81.5</td>
<td></td>
</tr>
<tr>
<td>Cultivars 160 35.3</td>
<td>78.9</td>
<td>88.3</td>
<td></td>
</tr>
<tr>
<td>Cultivars + tin (h.s.r.) 160 34.1</td>
<td>69.4</td>
<td>83.7</td>
<td></td>
</tr>
<tr>
<td>Cultivars + tin (h.s.r.) 160 38.5</td>
<td>71.8</td>
<td>84.1</td>
<td></td>
</tr>
<tr>
<td>l.s.d. (P = 0.05)A</td>
<td>n.s.</td>
<td>5.0</td>
<td>4.6</td>
</tr>
</tbody>
</table>

* Sowing rate × nitrogen fertiliser × tin gene interaction.

### Table 3. Percentage light interception of commercial cultivars and lines at different sowing rates

<table>
<thead>
<tr>
<th>M97</th>
<th>W97</th>
</tr>
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<tbody>
<tr>
<td>DC 15</td>
<td>DC 30</td>
</tr>
<tr>
<td>DC 65</td>
<td>W96</td>
</tr>
</tbody>
</table>

| Cultivars 150 | 49 | 38 | 73 | 54 |
| Cultivars + tin 150 | 48 | 44 | 70 | 56 |
| Cultivars 300 | 61 | 51 | 83 | 75 |
| Cultivars + tin 300 | 56 | 53 | 77 | 75 |
| l.s.d. (P = 0.05) | 4 | 10 | 6 | 6 |

### Fig. 4. Biomass of the cultivars and tin lines from sowing until ‘harvest ripe’ (DC 92) at C95.1, C95.2, C96N, and AP97. Error bars, standard errors; h.s.r., higher sowing rate; arrows indicate anthesis (DC 65).
C96N, the maximum number of shoots formed ranged from 350–1080 shoots/m² for freely tillering lines (averaged over all lines). Thus there were a much higher number of shoots formed (range 40–65%) in free tillering lines than in lines without the tin gene. This relationship was stronger in the Banks compared with the Kite near-isogenic pair and it is likely to be associated with the tin gene in several spring wheat genetic backgrounds resulting in a significant reduction in tiller number in accordance with earlier reported studies (Richards 1988; Duggan et al. 2002). Averaged over 4 genetic backgrounds in 6 experiments conducted over 3 years, Duggan et al. (2005) reported 11% fewer fertile spikes at maturity in lines with the tin gene compared with their near-isogenic free tillering counterparts. However, the maximum number of shoots formed in the near-isogenic contrasts is greater. At the 2 field sites where the highest and lowest maximum shoot numbers were recorded, namely AP97 and C96N, the maximum number of shoots formed ranged from 250–650 shoots/m² for lines with the tin gene to 350–1080 shoots/m² for freely tillering lines (averaged over all lines). Thus there were a much higher number of shoots formed (range 40–65%) in free tillering lines than in tin lines and a correspondingly higher mortality. A similar difference in tillering between lines with and without the tin gene was observed in the tube experiment. The reduction in tiller number associated with the tin gene was the result of an early cessation rather than a reduced rate of tillering. Earlier formed tillers are more likely to form fertile spikes (Rawson 1971) and so the suppression of the later formed tillers by the tin gene only has a small effect on spike number (Duggan et al. 2005). This may vary according to genetic background as in some cultivars, such as Banks, the tin gene appears to be suppressing tillers that otherwise may have gone on to produce fertile spikes. In contrast, the tin gene had minimal effect on the spike number of Bodallin, although as with other cultivars it did reduce the number of non-productive tillers produced (data not shown).

Stunting, which occurs when the stem fails to elongate and the plant eventually dies, associated with the presence of the tin gene in some environments (Atsmon et al. 1986; Duggan et al. 2002), was not observed in any of the field experiments reported here. Duggan et al. (2002) associated stunting in lines with the tin gene with a high photothermal quotient (PTQ) during early tillering. PTQ during tillering for late autumn–early winter sown wheat crops in Australia is at its lowest but then increases at the time of flowering in the spring. This is unlikely to stimulate a stunting response in lines containing the tin gene if they were to be released commercially in Australia. In environments where the PTQ is high shortly after sowing, such as in some northern hemisphere spring-sowing regions, stunting may be a potential concern and selection against stunting may be important.

### Table 4. Water-soluble carbohydrates (g/m²) in the stems at M97 (averaged across sowing rates) and AP97 (averaged across nitrogen treatments) at anthesis (DC 65) and maturity (DC 92) as well as the difference in levels of remobilisation

<table>
<thead>
<tr>
<th></th>
<th>Anthesis</th>
<th>Maturity</th>
<th>Anthesis – maturity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivars</td>
<td>368</td>
<td>65</td>
<td>303</td>
</tr>
<tr>
<td>Cultivars + tin</td>
<td>402</td>
<td>64</td>
<td>338</td>
</tr>
<tr>
<td>L.s.d. (P &lt; 0.05)</td>
<td>20</td>
<td>20</td>
<td>35</td>
</tr>
</tbody>
</table>

**Discussion**

**Tillering and spike number**

The presence of the tin gene in several spring wheat genetic backgrounds resulted in a significant reduction in tiller number in accordance with earlier reported studies (Richards 1988; Duggan et al. 2002). Averaged over 4 genetic backgrounds in 6 experiments conducted over 3 years, Duggan et al. (2005) reported 11% fewer fertile spikes at maturity in lines with the tin gene compared with their near-isogenic free tillering counterparts. However, the maximum number of shoots formed in the near-isogenic contrasts is greater. Associated with the increased weight of leaves was an increase in root weight up to the 5-leaf stage in tube-grown plants in the Banks background. This may occur as the developing plant is unable to allocate resources towards the production of new tillers and so a heavier root system results. This relationship was stronger in the Banks compared with the Kite near-isogenic pair and it is likely to be associated with greater tiller suppression associated with Banks. This increase in root weight was largely associated with seminal roots rather than nodal roots as few nodal roots had appeared at final harvest. It is not known whether nodal root growth of lines with the tin gene is affected. This may occur because of the close association between tiller appearance and nodal...
root growth (Klepper et al. 1984). The tin gene was also associated with a heavier spike weight at anthesis relative to total above-ground biomass (data not shown). This later translated into a higher harvest index and more grains per spike (Duggan et al. 2005).

Studies by Richards et al. (2002) showed that lines associated with the tin gene, unrelated to those reported here, also had higher water-soluble carbohydrate levels in the stems at anthesis. Increased partitioning to spikes and greater carbohydrate storage and remobilisation after anthesis, both of which were found here, should also contribute to the higher harvest index and kernel weight reported in the companion paper (Duggan et al. 2005).

Conclusions
Despite the tin gene almost halving the number of tillers formed in several different genetic backgrounds, it had little overall effect on the general growth and yield of wheat in field experiments in south-eastern Australia. There was a small decrease in light interception throughout crop growth associated with the tin gene, also occasional evidence of a reduction in biomass up to anthesis, evidence for greater storage of stem carbohydrates for translocation to the grain and for enhanced root growth. However, these small changes may be important to increase the yield of wheat where there is a terminal drought. For example, the small reduction in light interception may result in a small reduction in soil-water use before anthesis, which could later be used during grain filling (Passioura 1976; Richards and Townley-Smith 1987). This would result in an increased harvest index and grain weight, which is consistent with results reported by Duggan et al. (2005).

Results show little evidence that the tin gene is likely to disadvantage wheat yields unless sowing density is low or stunting (Duggan et al. 2002) is a problem. Both are unlikely in cereal-growing regions of Australia providing the sowing conditions are favourable. Low tillering lines grown here were unselected for yield and were contrasted with commercial wheat genotypes (from which they were derived) that were intensively selected for yield. Selection for yield among lines with the tin gene may further raise yields such that they could become superior to commercial wheats.

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