Developmental and growth controls of tillering and water-soluble carbohydrate accumulation in contrasting wheat (Triticum aestivum L.) genotypes: can we dissect them?

M. Fernanda Drecce1,*, Scott. C. Chapman2, Allan R. Rattey2, Jodi Neal2, Youhong Song3, John (Jack) T. Christopher4 and Matthew Reynolds5

1 CSIRO Plant Industry, Cooper Laboratory, PO Box 863, University of Queensland, Warrego Highway, Gatton, QLD 4343, Australia
2 CSIRO Plant Industry, Queensland Bioscience Precinct, 306 Carmody Road, St Lucia, QLD 4067, Australia
3 The University of Newcastle, School of Environmental and Life Sciences, Newcastle, NSW 2308, Australia
4 University of Queensland, Queensland Alliance for Agricultural and Food Innovation, Leslie Research Centre, 13 Holberton Street, Toowoomba, QLD 4350, Australia
5 CIMMYT, Int. Apdo. Postal 6–641, 06600 Mexico, DF, Mexico

* To whom correspondence should be addressed. E-mail: fernanda.drecce@csiro.au

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Abstract

In wheat, tillering and water-soluble carbohydrates (WSCs) in the stem are potential traits for adaptation to different environments and are of interest as targets for selective breeding. This study investigated the observation that a high stem WSC concentration (WSCc) is often related to low tillering. The proposition tested was that stem WSC accumulation is plant density dependent and could be an emergent property of tillering, whether driven by genotype or by environment. A small subset of recombinant inbred lines (RILs) contrasting for tillering was grown at different plant densities or on different sowing dates in multiple field experiments. Both tillering and WSCc were highly influenced by the environment, with a smaller, distinct genotypic component; the genotype-environment range covered 350–750 stems m⁻² and 25–210 mg g⁻¹ WSCc. Stem WSCc was inversely related to stem number m⁻², but genotypic rankings for stem WSCc persisted when RILs were compared at similar stem density. Low tillering–high WSCc RILs had similar leaf area index, larger individual leaves, and stems with larger internode cross-section and wall area when compared with high tillering–low WSCc RILs. The maximum number of stems per plant was positively associated with growth and relative growth rate per plant, tillering rate and duration, and also, in some treatments, with leaf appearance rate and final leaf number. A common threshold of the red:far red ratio (0.39–0.44; standard error of the difference=0.055) coincided with the maximum stem number per plant across genotypes and plant densities, and could be effectively used in crop simulation modelling as a ‘cut-off’ rule for tillering. The relationship between tillering, WSCc, and their component traits, as well as the possible implications for crop simulation and breeding, is discussed.

Key words: Nitrogen, plant density, sowing date, tillering, water-soluble carbohydrates, wheat (Triticum aestivum).

Introduction

Tillering and the accumulation of water-soluble carbohydrates (WSCs) in the stem are important traits that contribute to phenotypic plasticity in small-grain cereals. Tillering can be described as the degree of branching that determines the number
of spikes per unit area, an important yield component, also influencing light interception by the canopy and likely associated water use (e.g. Duggan et al., 2005a). To the extent that high tiller number contributes to early season ground cover, this trait assists in competing against weeds and minimizing water loss due to soil evaporation in Mediterranean climates (Borrass-Gelonch et al., 2010). The storage of stem WSCs, which upon mobilization to the grain can act as a buffer to declining photosynthetic capacity during grain filling, plays a role in the adaptation of wheat and barley to different environments. These WSCs, mainly fructans (Kuhbauch and Thome, 1989), can contribute to yield by as much as 50% under severe stress (see references in Blum, 1998; van Herwaarden et al., 1998a, b), and from 10% to 20% under non-stressed conditions (Gebbing and Schnyder, 1999; Shearman et al., 2005; Dreccer et al., 2009). Despite their roles in adaptation, little is known about the relationship between tillering and WSC accumulation, the potential impacts on the interpretation of the genotype×environment interaction (GEI), and the consequent utility of combining these traits as part of a breeding objective. This study is aimed at exploring the relationship between these traits.

Tiller number is regulated genotypically, but is also influenced by the environment. Genotypic variation in the degree of tillering expressed as a phenotype has been documented in wheat (Duggan et al., 2005b), rice (see Fujita et al., 2010, and references therein), and sorghum (Ferraris and Charles-Edwards, 1986a; Kim et al., 2010a). Various ‘domestication syndrome’ genes have been reported to regulate branching in Triticeae (Sakuma et al., 2011). Genes that regulate bud formation and growth have been described in maize, teosinte branched1 (tb1) which causes loss of apical dominance and promotes tillering (Doebly et al., 1997); in rice, Monoculm1 (MOC1) (Li et al., 2003) and LAX PANICLE2 (LAX2) (Tabuchi et al., 2011) inhibit axillary bud formation or growth; and in wheat, the tin (Spilmeyer and Richards, 2004) and tin3 (Kuraparthi et al., 2007) genes impinge on similar processes. While the effects of these single genes is known, consistent genetic differences in stem number per plant in diverse germplasm (Dreccer et al., 2008; Rebetzke et al., 2008) is frequently inherited quantitatively, and the relationship between quantitative trait loci (QTLs) involved in the regulation of productive tillers is a common focus of study (e.g. Deng et al., 2011; Naruoka et al., 2011).

Different processes underlie tiller initiation and outgrowth, cessation, senescence, and death (Table 1). Kirby et al. (1985) proposed that potential tiller number in wheat and barley was related to leaf initiation and appearance, followed a Fibonacci series, and was limited by a final leaf number. The timing of transition of the apex to the reproductive stage, which depends on the combination of genes regulating flowering time and the interaction with photoperiod and temperature, also influences tillering and C allocation (Hay and Kirby, 1991). The availability of carbohydrates for new tillers versus their demand was a key factor behind the final tiller number of wheat (Rodriguez et al., 1998), rice (Dingkuhn et al., 2006), and sorghum in different environments (Kim et al., 2010b) or across genotypes contrasting for tillering (Kim et al., 2010a). The maximum tiller number per plant and survival after the onset of stem elongation have been linked to an increased demand by main shoots with high leaf area in sorghum (van Oosterom et al., 2010b). Nutrient deficiency can have a direct impact on tiller initiation, as documented for P in wheat (Rodriguez et al., 1998) and barley (Prystupa et al., 2004). A decrease in the red:far red (R:FR) ratio as the canopy develops (Casal et al., 1986; Evers et al., 2006) and, to a lesser extent, a decrease in the amount of light reaching the soil (Evers et al., 2006) have been mentioned as triggers for cessation of tillering. A role for hormones in suppression/enhancement of bud growth

<table>
<thead>
<tr>
<th>Process</th>
<th>Phase</th>
<th>Initiation/appearance</th>
<th>Cessation</th>
<th>Senescence/death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Development</td>
<td>Leaf appearance rate (phylochron) (Kirby et al., 1985)</td>
<td>Start of stem elongation changes partitioning priority for photoassimilates (van Oosterom et al., 2011)</td>
<td>Internal competition for C or N (van Oosterom et al., 2010a)</td>
<td></td>
</tr>
<tr>
<td>C balance and partitioning</td>
<td>Supply/demand balance of photoassimilates per tiller (Kim et al., 2010a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light attributes</td>
<td>Coleoptile tiller (Peterson et al., 1982)</td>
<td>Auxin moving basipetally from the main apex and strigolactones moving acropetally from the roots suppress branching (McSteen, 2009)</td>
<td>Asynchrony in stem elongation (dominance) (McSteen, 2009)</td>
<td></td>
</tr>
<tr>
<td>Morphological characteristics and hormone-related control</td>
<td>Apex/organ morphology (dominance and related hormonal balance), movement of cytokinins from the roots promotes growth (McSteen, 2009)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Current knowledge on processes influencing tiller initiation, cessation, and death in cereals and selected examples from the literature.
in response to environmental factors and apical dominance has been advocated for (McSteen, 2009).

Stem WSC concentration (WSCc) in wheat is under complex genetic control, with many small effect QTLs (Rebetzke et al., 2008). Also in wheat, stem WSCc has been negatively correlated to tiller or spike number per unit area in genetically diverse germplasm (Tribio and Ollier, 1991; Dreccer et al., 2008, 2009; Rebetzke et al., 2008). In a previous report, Dreccer et al. (2009) found that a small set of recombinant inbred lines (RILs) from the Seri/Babax population (Olivares-Villegas et al., 2007), selected on their contrasting stem WSCc at anthesis, produced the same amount of biomass with fewer and heavier stems per m². This led to the hypothesis that higher WSC accumulation could be an emergent property of low tillering; that is, partitioning a similar amount of available carbon among fewer stems. This is consistent with the fact that the regulation of stem number per plant occurs earlier during crop development relative to stem WSC accumulation, which generally peaks after flowering (Ehdaie et al., 2006). If this hypothesis is correct, observed genotypic differences in WSCc accumulation would be dependent on stem/plant density. So far, most genotypic rankings of WSCc have been derived from trials sown at a single plant density (e.g. Rebetzke et al., 2008; Dreccer et al., 2009).

In this series of experiments in a subtropical summer rainfall environment, a small subset of RILs from the Seri/Babax population that had been shown to differ in WSCc in the stem and in stem number m⁻² when sown at a single density (150 plants m⁻²) (Dreccer et al., 2009; Rattey et al., 2009), was extended to include more lines contrasting in spike number at maturity (A. Rattey, personal communication). The underlying causes for observed differences in stem number per plant between RILs was investigated by studying leaf and tiller appearance, changes in light quality and quantity, as well as the accumulation of biomass, N, and WSCs. The hypothesis that the genotypic ranking of WSCc can vary with plant density was tested. A hypothetical model for the relationship between tillering and WSCc capturing the environmental and genotypic components is proposed, and the implications of the results for crop simulation models and breeding are discussed.

## Materials and methods

### Germplasm

The study used RILs from the Seri/Babax population described by Olivares-Villegas et al. (2007). The parental genotypes (based on released CIMMYT wheat lines) had been identified as differing in performance under drought while showing high yield potential. A set of six lines (‘Group-6’, Table 2) with similar height and anthesis, consisting of the two parents plus four RILs contrasting in WSCc (mg WSC g⁻¹ dry weight) in the stem+sheaths at anthesis, were tested at contrasting plant densities in 2007. Some of the Group-6 lines had also been tested and phenotyped in 2006 (Dreccer et al., 2009).

The set of lines evaluated in 2008 were the two parents plus 12 RILs (Group-14, Table 2) selected for their contrast in spike number m⁻² at physiological maturity, with a similar range in plant height and days to anthesis. These RILs had been examined in a range of environments at Gatton (Queensland, 27.55°S latitude 152.33°E longitude) where mean spike number m⁻² at physiological maturity had a 2-fold range (table 2 in Rattey et al., 2009). The lines were genotyped for major vernalization (VRN-A1, VRN-B1, and VRN-D1) and photoperiod (PPD-D1) genes and are classified as ‘spring wheats’ at all loci (Ben Trevaskis and Karen Cane, personal communication). In figures, genotypes are identified by initials (parents) or the three last numbers (RILs).

### Treatments and trials

Multiple field and rainout shelter trials were conducted with some or all of the lines described in Table 2, manipulating plant density (to test the hypothesis of density-dependent WSCc accumulation) or watering and sowing dates (normal and late) to create a range of contrasting environmental conditions for WSCc accumulation and tillering. The main site was CSIRO’s field station in Gatton, and a grower’s field at Dalby (Queensland, 27.19°S latitude, 151.21°E longitude) (Table 3). On sowing dates within the optimal period for yield potential (early May to mid June), current local practice for rainfed environments in the region is to sow at 100 plants m⁻². In 2008 on the normal sowing date, plant populations were 50 (very low), 100 (low), or 400 (high) plants m⁻², while at late sowing, targets were 180 (low) and 300 (high) plants m⁻². As per common farm practice for late sowing, the range of density explored was narrower; the low density was higher than at normal sowing to achieve comparable cover in a shorter growing period, and the high density was lower to avoid overcrowding small plants. In 2006, trials were grown combining water availability and N level, but N supply was not limiting at the lower dosage (for details, see Dreccer et al., 2009). Trials were either randomized block designs with a single factor (genotype) or factorials (e.g. genotype x density) with a row–column design, all with three or four replicates. Experimental plots were seven rows wide, at 0.25 m inter-row distance (or 0.20 m in 2006) and 8 m long, except in the rainout shelter trials where plots were 5 m long. Prior to sowing, individual grain weights of seed sources were used to calculate seed weight per plot, with the objective of establishing the same plant density across genotypes. The soils were Black Vertosols which can hold up to 250 mm of plant available water over the maximum root depth of 180 cm. Plots were maintained weed, pest, and disease free, and nutrients were non-limiting. N was added as urea in one basal fertilization pre-sowing, after soil analysis, aiming for 250–300 kgN ha⁻¹. Weather data were measured at the trials, except for radiation data which were downloaded from the local meteorological stations (Australian Bureau of Meteorology, SILO patch-point data set, www.bom.gov.au/silo, within ±1 km radius of the site). In 2008, soil temperature was monitored until the end of tillering using a 15 cm long probe buried horizontally at 4–5 cm below the surface, approximately the position of the tillering node before stem elongation.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>WSCc score</th>
<th>Tillering score</th>
<th>Group-6</th>
<th>Group-14</th>
</tr>
</thead>
<tbody>
<tr>
<td>SB003</td>
<td>Low</td>
<td>High</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>SB010</td>
<td>Low</td>
<td>High</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>SB071</td>
<td>High</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>SB183</td>
<td>High</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>SB165</td>
<td>High</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>SB062</td>
<td>High</td>
<td>Low</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>SB101</td>
<td>Low</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>SB109</td>
<td>Low</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>SB155</td>
<td>Low</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>SB159</td>
<td>Low</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>SB160</td>
<td>High</td>
<td>Low</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>SB172</td>
<td>Low</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Seri</td>
<td>Parent</td>
<td>Parent</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Babax</td>
<td>Parent</td>
<td>Parent</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Table 2. Prior classification of recombinant inbred lines from the Seri/Babax population for WSCc and tillering. See the Materials and Methods for an explanation of the classification.
Table 3. Trials, treatments and seasonal weather.

<table>
<thead>
<tr>
<th>Trial code</th>
<th>Environment</th>
<th>Sowing date</th>
<th>Genotypes</th>
<th>Density (plants m⁻²)</th>
<th>PAR (MJ m⁻² d⁻¹)⁰</th>
<th>Temperature (°Cd)b</th>
<th>In crop rainfall+irrigation (mm)²</th>
</tr>
</thead>
<tbody>
<tr>
<td>05NRFED_lowN</td>
<td>Rainfed Gatton</td>
<td>6 June 2005</td>
<td>SB003, SB010, SB062, SB169</td>
<td>150</td>
<td>16.3 (3.5)</td>
<td>16.1 (24.0–8.2)</td>
<td>150 (20.0–9.0–10)</td>
</tr>
<tr>
<td>05NRFED_highN</td>
<td>Rainfed Gatton</td>
<td>6 June 2005</td>
<td>SB003, SB010, SB062, SB169</td>
<td>150</td>
<td>16.3 (3.5)</td>
<td>16.1 (24.0–8.2)</td>
<td>150 (20.0–9.0–10)</td>
</tr>
<tr>
<td>05NIRR_lowN</td>
<td>Rainfed Gatton</td>
<td>7 June 2007</td>
<td>SB003, SB010, SB062, SB169</td>
<td>150</td>
<td>16.3 (3.5)</td>
<td>16.1 (24.0–8.2)</td>
<td>150 (20.0–9.0–10)</td>
</tr>
<tr>
<td>05NIRR_highN</td>
<td>Rainfed Gatton</td>
<td>7 June 2007</td>
<td>SB003, SB010, SB062, SB169</td>
<td>150</td>
<td>16.3 (3.5)</td>
<td>16.1 (24.0–8.2)</td>
<td>150 (20.0–9.0–10)</td>
</tr>
<tr>
<td>05ROS-1SD</td>
<td>Gatton irrigated, late sowing</td>
<td>17 July 2005</td>
<td>SB003, SB010, SB062, SB169</td>
<td>150</td>
<td>16.3 (3.5)</td>
<td>16.1 (24.0–8.2)</td>
<td>150 (20.0–9.0–10)</td>
</tr>
<tr>
<td>05ROS-2SD</td>
<td>Gatton irrigated, late sowing</td>
<td>17 July 2005</td>
<td>SB003, SB010, SB062, SB169</td>
<td>150</td>
<td>16.3 (3.5)</td>
<td>16.1 (24.0–8.2)</td>
<td>150 (20.0–9.0–10)</td>
</tr>
<tr>
<td>07SBGTS-2</td>
<td>Gatton irrigated</td>
<td>18 May 2007</td>
<td>Group-6</td>
<td>100</td>
<td>16.3 (3.5)</td>
<td>16.1 (24.0–8.2)</td>
<td>150 (20.0–9.0–10)</td>
</tr>
<tr>
<td>07SBGTS-3</td>
<td>Gatton irrigated</td>
<td>22 May 2007</td>
<td>Group-6</td>
<td>100</td>
<td>16.3 (3.5)</td>
<td>16.1 (24.0–8.2)</td>
<td>150 (20.0–9.0–10)</td>
</tr>
<tr>
<td>07SBGTS-4</td>
<td>Gatton irrigated</td>
<td>22 May 2007</td>
<td>Group-6</td>
<td>100</td>
<td>16.3 (3.5)</td>
<td>16.1 (24.0–8.2)</td>
<td>150 (20.0–9.0–10)</td>
</tr>
<tr>
<td>07SBGTS-5</td>
<td>Gatton irrigated</td>
<td>22 May 2007</td>
<td>Group-6</td>
<td>100</td>
<td>16.3 (3.5)</td>
<td>16.1 (24.0–8.2)</td>
<td>150 (20.0–9.0–10)</td>
</tr>
<tr>
<td>08SBTill-12</td>
<td>Gatton irrigated, normal sowing</td>
<td>29 May 2008</td>
<td>Group-14</td>
<td>50, 100</td>
<td>16.3 (3.5)</td>
<td>16.1 (24.0–8.2)</td>
<td>150 (20.0–9.0–10)</td>
</tr>
<tr>
<td>08SBTill-4²</td>
<td>Gatton irrigated, late sowing</td>
<td>15 August 2008</td>
<td>Group-14</td>
<td>180, 300</td>
<td>16.3 (3.5)</td>
<td>16.1 (24.0–8.2)</td>
<td>150 (20.0–9.0–10)</td>
</tr>
</tbody>
</table>

Notes:

- a Seasonal average and standard deviation (in parentheses)
- b Seasonal average, and average maximum and minimum temperature in parentheses.
- c Initial plant available water was between 200 and 250 mm.
- d Maturity sampling lost due to hailstorm.

Measurements

Phenology, leaf and tiller appearance: Phenological stages were recorded using the decimal code (DC) of Zadoks et al. (1974), with anthesis diagnosed when 50% of the spikes had anthers extruded (DC65). The dynamics of leaf appearance and tillering were followed in four tagged plants per plot of Group-6 in 2008. The green tiller number per plant was counted and the Haun index (Haun, 1973) recorded twice a week until anthesis. Tillers were counted as soon as they were visible, 5–10 mm long, and were considered senescent when the last expanding leaf was ≥50% yellow. The phyllochron was calculated as the inverse of the slope of the Haun index and cumulative thermal time, from advanced leaf 2 to the flag or penultimate leaf. In 2008, daily thermal time was calculated based on 5 h estimates, as temperature was recorded within the trial every 5 min. Thermal time accumulation was estimated using the APSIM wheat model’s cardinal temperatures linked by linear interpolation (Tbase=0 °C, Toptimum=26 °C, and 34 °C; http://www.apsim.info/wiki/Wheat.ashx). This method assumes that cardinal temperatures are genotype independent. In 2008, soil temperature was the input while the apex was below ground (DC30), and air temperature was the input thereafter. The green stem number per plant was plotted against leaf number, calculated as the ratio between thermal time and the phyllochron, with the aim of obtaining a developmental index independent of differences in the phyllochron due to the treatments (as in Evers et al., 2006).

To plot the number of green stems (tillers+main stem) per plant (y) versus leaf number or thermal time from emergence (x), broken line regressions up to the end of the plateau (x₂, y₂ in Fig. 1) were fitted using the segmented function of the ‘Segmented’ software package (Muggeo, 2008) within the R statistical language software (R Development Core Team, 2008) (Fig. 1). ‘Obj’ was set as a basic linear model ‘lm(y~1)’, and ‘seg.Z’ as ‘-x’ to force a flat plateau. The maximum stem number per plant (y₃) could be calculated as:

\[ y₃ = k₂ × (x₃ - x₀) + k₀ × x₀ \] (1)

where \( y₂ = \frac{w₂ - w₁}{Tacc₂ - Tacc₁} \) and \( y₃ = \frac{w₃ - w₁}{Tacc₃ - Tacc₁} \), with \( w₂ \) and \( w₃ \) being the seed weight (as surrogate for plant weight at emergence), and \( Tacc₂ \) and \( Tacc₃ \) the thermal time (°Cd) accumulated from emergence (Tb=0 °C) to the end of tillering (Tacc,2).

The relative growth rate (RGR, °Cd⁻¹) represents the biomass gain per unit of existing biomass and per unit thermal time and was calculated as:

\[ RGR = \frac{\ln(w₂) - \ln(w₁)}{Tacc₂ - Tacc₁} \] (3)

k₀ and k₁ are tillering rates. As tiller mortality occurred in a very short period, involving few observation dates, it was not possible to fit a segmented function to the whole data set. Hence, data from x₂, y₂ onwards (Fig. 1) were fit to a bilinear model using GenStat 10th Edition (VSN International, Hemel Hempstead, UK). Given the scarcity of data points in the tiller death phase, a comparison between senescence rates (k₃) among treatments is not attempted. The final tiller number per plant is y₃.

Biomass, WSC, and N: Borderline biomass cuts were made at the stage of 5–6 leaves emerged, end of tillering (DC30–DC31), booting, anthesis (or 100 °Cd after anthesis in 2006), and maturity, depending on the trial. Maturity cuts were of 0.250 m² or 0.375 m². Stems and spikes were counted, plants were separated into organs (stem, spike, leaf, senesced [≥50% yellow leaf]), green leaf area was measured at anthesis, organs were dried at 70 °C for 3 days and weighed, and the harvest index was calculated on a dry weight basis at maturity. Height to the top of the spike (excluding awns) was measured in the standing crop shortly after anthesis. Individual grain weight from the maturity quadrat harvest was calculated from the average of three lots of 100 grains.

The growth rate per plant (GR, g °Cd⁻¹ plant⁻¹) was calculated as:

\[ GR = \frac{w₂ - w₁}{Tacc₂ - Tacc₁} \] (2)

where \( w₂ \) was biomass plant⁻¹ at the end of tillering (DC30–DC31), \( w₁ \) was the seed weight (as surrogate for plant weight at emergence), and \( Tacc₂ \) the thermal time (°Cd) accumulated from emergence (Tb=0 °C) to the end of tillering (Tacc,2).

The relative growth rate (RGR, °Cd⁻¹) represents the biomass gain per unit of existing biomass and per unit thermal time and was calculated as:

\[ RGR = \frac{\ln(w₂) - \ln(w₁)}{Tacc₂ - Tacc₁} \] (3)
The levels of WSCs in the stems plus sheaths (referred to as ‘stems’ throughout the text) at anthesis were determined by sequential extraction of the ground tissue in 80% ethanol and water (for details, see van Herwaarden et al., 1998a) followed by determination in the extract using the anthrone method of Yemm and Willis (1954) with fructose as the standard. Nitrogen was determined from 5–6 leaves to maturity in green leaves, stems, and spikes by combustion analysis using oxygen at 1100 °C and EDTA as a calibrant (CNS-2000 Combustion Analyser, LECO, St Joseph, MI, USA).

Light interception and quality The fraction of photosynthetically active radiation (PAR) intercepted was calculated from measurements taken between 11:00h and 13:00h with a ceptometer (AccuPAR, Decagon Devices Inc, Pullman, WA, USA) above the canopy and immediately below the level of green leaf. Data were fitted to a logistic curve using GenStat 10th Edition (VSN International).

\[ \text{Fraction PAR intercepted} = C \left(1 + e^{-b(x-M)} \right) \]  

C is the maximum fraction of intercepted radiation, M the thermal time at which the increase in the fraction of PAR interception is the greatest, and b is the maximum rate of change in interception on M.

In the 2008 trials at normal sowing, the R:FR light ratio was measured with a two-channel sensor with narrow band filters centred at 660 nm and 730 nm (Skye Instruments Ltd, Powys, UK) between 240 °Cd and 880 °Cd after emergence. Data were fitted to thermal time after emergence using a critical exponential curve in GenStat 10th Edition (VSN International).

Characterization of stems and leaves For each leaf on the main stem, leaf area and auricle height (distance from the base of the plant) was measured in successive harvests in the 08SBTiller-12 trial. In 07SBDens-5, main stems or primary tillers were collected and cross-sectioned 1 cm below the level of green leaf. Data were fitted to a logistic curve using GenStat 10th Edition (VSN International).

Data analyses

Data were analysed following the two-stage protocol described by Welham et al. (2006) and Rattey and Shorter (2010) to apply a mixed-model method to the estimation of multienvironment effects. In the first stage, best linear unbiased estimates (BLUEs) were obtained for each genotype×environment combination using the ASReml software package (Gilmour et al., 2009) running in the R operating system (R Development Core Team, 2008) and incorporating appropriate modelling of field spatial trends (Gilmour et al., 1997). Best linear unbiased predictors (BLUPs) were calculated for each genotype within by applying REML analyses across environments (considered as fixed effects). The inputs to this analysis were the single environment BLUEs and weights using the inverse of the standard errors for each BLUE (Smith et al., 2001). In the second stage, to estimate across environment effects, the interactions of genotype and environment were estimated as random effects using either uniform correlation (corh) or diagonal (diag) variance models (for details, see Rattey and Shorter, 2010).

Results

Weather, yield, and components across trials

Average PAR from sowing to maturity varied from 15.0 to 20.0 MJ m⁻² d⁻¹ across Gatton trials, with higher radiation in later sowings; a similar trend was observed for average temperature (Table 3).

Compared with high tillering (HT) lines, low tillering (LT) lines from Group-6 (SB062 and SB169) flowered ~2.3 d earlier (SE=0.44), had slightly better yield via similar biomass and higher harvest index, lower grain number m⁻², fewer spikes m⁻², higher grain number per spike, and higher individual grain weight (Table 4). While total biomass and leaf area index (LAI) at anthesis were similar among lines, LT lines had slightly lower crop N uptake at anthesis, though there were no genotypic trends by maturity (Table 4).

Tillering dynamics differ in high and low tillering lines

The appearance, cessation, and death of stems per plant were studied in detail for the Group-6 lines in 2008, with the dynamics described in Fig. 1. The regression models fitted to each treatment were highly significant (P < 0.001), with R² >0.95, for both the segmented and the bilinear functions (see the Materials and methods).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Tilleting score</th>
<th>Yield (g m⁻²)</th>
<th>Biomass at maturity (g m⁻²)</th>
<th>HI</th>
<th>Grain number (per m²)</th>
<th>Grain weight (mg grain⁻¹)</th>
<th>Spike number (per m²)</th>
<th>Grains per spike</th>
<th>Height (cm)</th>
<th>Biomass at anthesis (g m⁻²)</th>
<th>LAI at anthesis (m² m⁻²)</th>
<th>Crop N at anthesis (g m⁻²)</th>
<th>Crop N at maturity (g N m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SB003</td>
<td>High</td>
<td>558</td>
<td>1277</td>
<td>0.43</td>
<td>16 134</td>
<td>32.3</td>
<td>445</td>
<td>38.5</td>
<td>81.2</td>
<td>785.6</td>
<td>3.2</td>
<td>15.5</td>
<td>21.6</td>
</tr>
<tr>
<td>SB010</td>
<td>High</td>
<td>563</td>
<td>1263</td>
<td>0.44</td>
<td>16 571</td>
<td>33.1</td>
<td>440</td>
<td>40.9</td>
<td>76.7</td>
<td>752.5</td>
<td>3.7</td>
<td>15.6</td>
<td>21.3</td>
</tr>
<tr>
<td>SB062</td>
<td>Low</td>
<td>575</td>
<td>1273</td>
<td>0.46</td>
<td>15 001</td>
<td>39.2</td>
<td>360</td>
<td>41.7</td>
<td>81.2</td>
<td>741.9</td>
<td>3.0</td>
<td>15.2</td>
<td>20.3</td>
</tr>
<tr>
<td>SB169</td>
<td>Low</td>
<td>564</td>
<td>1276</td>
<td>0.46</td>
<td>15 820</td>
<td>38.0</td>
<td>364</td>
<td>41.8</td>
<td>84.1</td>
<td>781.8</td>
<td>3.2</td>
<td>15.2</td>
<td>21.8</td>
</tr>
<tr>
<td>Seni</td>
<td>Parent</td>
<td>570</td>
<td>1275</td>
<td>0.46</td>
<td>15 795</td>
<td>35.8</td>
<td>401</td>
<td>41.3</td>
<td>80.6</td>
<td>764.8</td>
<td>3.1</td>
<td>15.2</td>
<td>21.3</td>
</tr>
<tr>
<td>Babax</td>
<td>Parent</td>
<td>564</td>
<td>1278</td>
<td>0.43</td>
<td>15 319</td>
<td>36.5</td>
<td>394</td>
<td>40.9</td>
<td>84.4</td>
<td>801.5</td>
<td>3.6</td>
<td>15.5</td>
<td>21.4</td>
</tr>
<tr>
<td>SED</td>
<td></td>
<td>3.4</td>
<td>3.3</td>
<td>0.006</td>
<td>392</td>
<td>1.6</td>
<td>21</td>
<td>0.7</td>
<td>1.7</td>
<td>78.9</td>
<td>0.8</td>
<td>0.1</td>
<td>0.3</td>
</tr>
</tbody>
</table>

a According to Table 2.
Sowing date, density, and genotype had an impact on final stem number per plant ($y_3$). On average, early sown crops had more final stems per plant and a greater absolute impact of sowing density than the late ones (Fig. 2; Supplementary Fig. S1 and Supplementary Table S1 available at JXB online). For those treatments where there was tiller senescence, final stem number...
per plant ($y_3$) was associated with the maximum stem number ($y_1$) ($y_3=0.8234y_1–1.1843, R^2=0.96, P < 0.001, n = 12$). On the normal sowing date, the maximum stem number per plant was 0.4–2.0 stems lower on average in the LT versus the HT lines, at high and low density, respectively; similar to the difference in final tiller number ($y_3$) for the same treatments. In the more extreme environment of late planting, genotypic differences were less consistent, for instance SB003 (HT) and SB062 (LT) achieved a very similar final stem number per plant at a given density.

The tillering appearance phase was characterized by two different rates of increase, $k_0$ and $k_1$ in most genotypes on the normal sowing date at low and very low density, whereas a single slope ($k_1$) described any other treatments. While tiller categories were not differentiated at counting, it is very likely that primary tillers are represented by slope $k_0$ for those treatments with two slopes (e.g. very low density) and $k_1$ for those treatments with only one slope (e.g. high density). The tiller appearance rates differed based on leaf number versus thermal time, mainly due to the longer phyllochron at high density and the difference between sowing dates (Fig. 2a–f; Supplementary Fig. S1a–f at JXB online). The shift from $k_0$ to $k_1$ (very low and low density) or from $k_1$ to a plateau (high density) occurred around physiological age 4±0.85 (SE) (i.e. node=4) for the normal sowing date and 5.4±0.6 (SE) for the late sowing date (Fig. 2g–l). LT lines had a lower $k_1$, ~70–80% of that in HT lines for the normal sowing date, and ~90% for the late sowing date. The thermal time to reach the plateau ($x_1$) tended to be shorter in LT lines, but differences were not significant (Supplementary Table S1 at JXB online). The maximum tiller number per plant ($y_1$) was associated with the tillering rate per leaf ($k_1$) ($y=2.84x+1.25, R^2=0.82, P < 0.001, n=30$) and the duration ($x_1$) in thermal time ($y=0.0463x–17.1, R^2=0.90, P < 0.001, n=30$).

Tillering window: potential limits by phyllochron, final leaf number, and phenology

The phyllochron and the final leaf number set an upper limit to the number of tillers per plant. Linear regressions were fitted per replicate to Group-6 lines in 2008 to calculate the phyllochron, and were highly significant ($P < 0.001, R^2 > 0.99$). There was no consistently significant genotypic impact on the phyllochron, but LT lines had a trend towards lower final leaf number (Fig. 3a, b). Plant density had a significant and opposite effect on the phyllochron and final leaf number (Fig. 3a, b), High density resulted in a significantly longer phyllochron but consistently lower leaf number across sowing dates and genotypes.

The start of stem elongation (one node detectable, DC31) and flowering are phenological hallmarks for different aspects of tillering as they represent a swift change in resource allocation within the plant. In the 2008 trials, a Zadoks evaluation at the start of stem elongation showed that Group-6 HT lines were ~0.25–0.5 nodes younger than LT lines, in the normal and late planting, respectively, though differences were not significant;
plant density did not have a significant effect on node number on this date (data not shown). The LT group tended to flower ~1–3 d earlier than the HT group, in line with the rest of the trials. The slightly earlier flowering in the Group-6 LT lines was associated with the aforementioned slightly lower final leaf number, less than half a leaf on average (~0.4) (Fig. 3a, b).

The thermal time to flowering was significantly shortened in the late sowing by ~250 °Cd ($P < 0.001$) (Fig. 3c), suggesting sensitivity to photoperiod or an interaction between these factors and higher temperatures, despite the fact that the lines were characterized as photoperiod insensitive for a known major gene. A late sowing resulted in a shorter phyllochron ($P=0.002$) and a lower final leaf number ($P < 0.001$) (Fig. 3a, b). An analysis across the Group-14 genotypes on the normal sowing date indicated that the thermal time to anthesis was longer in the very low compared with the other densities ($P < 0.001$) (Fig. 3d), equivalent to a 1–1.5 d delay. The larger number of tillers of different categories at this density could make the diagnosis of phenology at the canopy level more difficult.

Tillering cessation: light interception and R:FR ratio at maximum tiller number

In 2008, the evolution of light interception (Table 5; Supplementary Fig. S2 at JXB online) and R:FR ratio (data not shown) was followed at normal sowing. In both cases, fitted curves had $R^2 \geq 0.955$ and $P < 0.001$. All treatments were at 0.98–0.99 PAR interception by DC30 (800–830 °Cd) or earlier; the increase in the fraction of PAR intercepted with thermal time was markedly affected by plant density. The thermal time to maximum rate of increase in interception ($M$; Equation 4) was reached earlier the higher the density. The LT line SB169 generally achieved full interception faster than other genotypes. The green LAI at anthesis was significantly different among sowing dates ($P < 0.001$) and densities ($P < 0.001$), genotypic differences were not clear (normal sowing, SB003=5.5, SB10=6.0, SB062=5.2, SB169=5.4, Seri=5.2, Babax=5.6; late sowing, SB003=3.8, SB10=4.7, SB062=3.1, SB169=3.4, Seri=3.2, Babax=3.8; within sowing date LSD$_{0.05}$=0.9).

The fraction of PAR intercepted at cessation of tiller appearance (maximum stem number) varied from 0.77 to 0.92 across all densities [standard error of the difference (SED)=0.0788]; however, genotypic differences were not consistent across densities or correspond to tillering grouping within a particular density (Fig. 4). The R:FR value at maximum tiller number varied between 0.39, 0.44, and 0.40 on average at the very low, low, and high density, respectively. These values were not significantly different (SED=0.055) and there was no clear genotypic effect.

Morphological differences between high and low tillering lines

A comparison of stem characteristics in the penultimate internode showed that HT lines had a smaller cross-section, lower total wall area, but not necessarily thinner walls at that particular upper internode (Fig. 5). The leaf area of individual leaves on the main stem of Babax was generally larger than on that of Seri from leaf 6 onwards, but similar to or lower than that of SB169, while SB10 had smaller leaves (Fig. 6). The first six leaves tended to have a larger area at high density, and subsequent leaves were larger at low density. The maximum stem number per plant was negatively related to the average of the individual size of all leaves in the main shoot, more significantly at low and very low density ($y=24.55–0.73x$, $R^2=0.80$, $P < 0.001$, $n=12$) than at high density ($y=4.94–0.08x$, $R^2=0.67$, $P=0.043$, $n=6$). At high density, genotypic differences in individual leaf area were larger, leaves were thinner (data not presented), and distance from the base of the plant to the auricle of each leaf

### Table 5

Parameters of the fraction of PAR intercepted curve (Equation 4) and their standard error ($n=8$ or 9) The maximum fraction intercepted ($C$) was not significantly different from 1 in all treatments. The curve is illustrated in Supplementary Fig. S2 at JXB online.

<table>
<thead>
<tr>
<th>Trial code</th>
<th>Density</th>
<th>Genotype</th>
<th>Tillering score$^a$</th>
<th>b (°Cd$^{-1}$)</th>
<th>M (°Cd)</th>
<th>$R^2$</th>
<th>F-prob</th>
</tr>
</thead>
<tbody>
<tr>
<td>08SBTiller-12</td>
<td>Very low</td>
<td>SB003</td>
<td>High</td>
<td>0.010 (0.001)</td>
<td>516 (15)</td>
<td>99.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SB10</td>
<td>High</td>
<td>0.011 (0.001)</td>
<td>502 (14)</td>
<td>99.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SB062</td>
<td>Low</td>
<td>0.012 (0.001)</td>
<td>530 (11)</td>
<td>99.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SB169</td>
<td>Low</td>
<td>0.012 (0.001)</td>
<td>471 (16)</td>
<td>99.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Seri</td>
<td>Parent</td>
<td>0.012 (0.001)</td>
<td>520 (15)</td>
<td>99.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Babax</td>
<td>Parent</td>
<td>0.012 (0.001)</td>
<td>496 (15)</td>
<td>99.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>SB003</td>
<td>High</td>
<td>0.014 (0.001)</td>
<td>434 (9)</td>
<td>99.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SB10</td>
<td>High</td>
<td>0.012 (0.001)</td>
<td>454 (13)</td>
<td>99.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SB062</td>
<td>Low</td>
<td>0.014 (0.002)</td>
<td>431 (15)</td>
<td>99.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SB169</td>
<td>Low</td>
<td>0.013 (0.002)</td>
<td>406 (12)</td>
<td>99.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Seri</td>
<td>Parent</td>
<td>0.013 (0.002)</td>
<td>430 (13)</td>
<td>99.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Babax</td>
<td>Parent</td>
<td>0.014 (0.002)</td>
<td>420 (14)</td>
<td>99.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>SB003</td>
<td>High</td>
<td>0.016 (0.001)</td>
<td>293 (5)</td>
<td>99.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SB10</td>
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<td>0.015 (0.003)</td>
<td>325 (8)</td>
<td>98.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SB062</td>
<td>Low</td>
<td>0.014 (0.002)</td>
<td>324 (8)</td>
<td>98.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SB169</td>
<td>Low</td>
<td>0.014 (0.002)</td>
<td>293 (9)</td>
<td>97.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Seri</td>
<td>Parent</td>
<td>0.016 (0.003)</td>
<td>327 (8)</td>
<td>98.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Babax</td>
<td>Parent</td>
<td>0.016 (0.003)</td>
<td>318 (9)</td>
<td>97.2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

$^a$ According to Table 2.
position longer (e.g. auricle height of leaf 6 at normal sowing: very low density=10.8 cm, low=13.4 cm, high=18.2 cm, SE=0.48).

WSCc was inversely related to stem number but genotypic rankings for stem WSCc persist when compared at similar stem density.

In order to compare genotypic rankings for stem WSCc versus stem number m$^{-2}$ at anthesis across trials, these variables were standardized with respect to the trial mean (see Materials and methods) (Fig. 7a). The HT lines consistently clustered as having low WSCc compared with the LT lines, which clustered as high WSCc across all trials. When the stem number m$^{-2}$ was similar to the trial mean (standardized value ~1), the LT lines generally had a higher stem N concentration (Nc) than the trial mean (Fig. 7b). The parental lines did not differ significantly in stem number m$^{-2}$ but had contrasting stem WSCc and Nc, Seri presenting higher stem WSCc and lower stem Nc than Babax (Fig. 7a, b).

These relationships are further illustrated in Fig. 7c and 7d with 2008 trials as they had more genotypes (Group-14). Within the stem density range between 450 and 600 stems m$^{-2}$, lines classified as LT had higher WSCc than their counterparts (LT=121, HT= 67.8, parents=95.9 mg g$^{-1}$, LSD$_{0.05}=33.6$); on average, the stem WSCc was higher in the normal than in the late planting (blue and red symbols in Fig. 7c). Genotypic differences in WSCc were not linked to differences in total biomass at the start of stem elongation or anthesis (data not presented) and only weakly to the stem biomass at anthesis \[WSCc (mg g^{-1})=6.9+0.179 \text{ stem biomass (g m}^{-2}), R^2=0.21, P < 0.013\]. However, WSCc was highly correlated to the actual amount of WSC \[WSC (g m^{-2})=0.69 WSCc^{-19.3}, R^2=0.87, P < 0.001\].

**Fig. 4.** R:FR ratio versus the fraction of PAR intercepted at maximum green stem number per plant. Vertical and horizontal bars are standard errors. Data from 08SBTiller-12, normal sowing. Each panel represents a different sowing density.
Despite the differences in stem number and stem WSCc or Nc among genotypes contrasting in tillering across all trials, at the whole crop level, the changes in Nc as a function of biomass could be described by a single equation (Fig. 8a, b). Among the factors contributing to the single relationship at the crop level is leaf Nc, which was slightly but not significantly higher in LT than in HT lines across all trials (4.13 versus 4.07, respectively, SE=0.03), opposite to the trend observed in Nc in stems. The specific leaf N (SLN; gN in green leaves m^{-2} leaf) was 1–7% higher in the LT lines in 2008 (late and normal sowing, respectively); the differences were not statistically significant. An analysis of SLN at anthesis across all trials (2006–2008) in Group-6 showed that the LT lines had on average 7% higher SLN than the HT lines (2.2 versus 2.0 gN m^{-2}, LSD=0.23), although differences were not significant.

**Maximum stem number in relation to leaf appearance rate, final leaf number, and growth rate**

The maximum stem number per plant was positively related to leaf appearance rate (inverse of the phyllochron) within each sowing date, except at very low density (Fig. 9a), and to the final leaf number per main stem at low densities on both sowing dates (Fig. 9b). It was also positively associated with increases in biomass gain per plant or per unit biomass between emergence and end of tillering (Fig. 9c, d).

**Discussion**

**Tillering: genotypic and environmental drivers revisited**

Tillering showed a strong genotypic component but was highly responsive to environmental conditions. A number of mechanisms have been proposed to regulate the maximum stem number per plant. Kirby et al. (1985) suggested that genetic variation in the leaf appearance rate could be used to predict and select for different tillering patterns. In the current study, the phyllochron (inverse of the leaf appearance rate) showed little or no genetic variation, but was shorter in the late than in the normal sowing date plants and longer at high density (Fig. 3a). This led to a positive response of maximum stem number per plant to leaf appearance rate within each sowing date, as predicted by Kirby and colleagues (Fig. 9a). Differences between sowing dates at low density were consolidated in one relationship when final leaf number was used as the predictive variable (Fig. 9b). In a barley double haploid population with limited variation in the phyllochron, Borras et al. (2009) also found significant genetic correlations between maximum tiller number per plant and final leaf number. Similar to this study, they found that the maximum tiller number was related to both tillering rate and duration. In a follow-up study with a wheat population, there was no association between the maximum number of tillers and the timing of tillering cessation (Borras-Gelonch et al., 2012). These studies highlight that different processes may be important for tillering and are worth accounting for in simulation modelling aimed at reproducing GEI, but genetic variation for component traits is population specific. Furthermore, component traits may compensate, for example if the phyllochron is short (more leaves can appear in a given time) but so is the time to double ridge. Some of these relationships are illustrated in the hypothetical diagram of Fig. 10. Results from the current study contrast partly with those reported in sorghum, where increased leaf appearance rate and increased individual leaf size were related to low tillering, and the inference was made that increased early vigour of the main shoot negatively affected other processes that demand carbon (van Oosterom et al., 2011). It is interesting to...
note that in sorghum and maize, tillers are principally produced on the main stem, while genotypic variation for secondary tillering may account for a large proportion of the maximum tiller number in wheat. Overall, the absolute or relative growth rate between emergence and end of tillering was a better predictor of maximum stem number per plant across treatments than leaf appearance rate or final leaf number (Fig. 9c, d). This seeming causality could be partly underpinned by an iterative process where each new tiller can also boost growth. Proving this point is beyond the scope of this study; hence, only the influence of growth rate on stem number was captured in Fig. 10. An association between crop growth rate or supply/demand ratio and

Fig. 6. Individual leaf area for the Group-6 genotypes at Very Low, Low, and High density at normal sowing (08SBTiller-12). Vertical bars are ±SE. Bilinear regressions have been fit to SB010 and SB169 from leaf 2 onwards. Observations at Very Low density were only taken till leaf 7.
maximum tiller number has also been found in sorghum (Kim et al., 2010b) and rice (Luquet et al., 2006).

An important contribution from the present study came from comparing extreme densities. The maximum stem number per plant varied with the genotype in a narrow range of final leaf number at very low density, possibly due to the greater contribution of higher order tillering cohorts. At high density, the maximum stem number did not respond to final leaf number, probably reflecting a different constraint, light quality. The R:FR ratio at maximum tiller number was similar across genotypes and densities (0.39–0.44; SED=0.0546), supporting the range reported by Evers et al. (2006) (0.35–0.40). These authors suggested a positive, albeit weaker, association between the fraction of intercepted radiation, ~0.5–0.6, and the cessation of tillering. In comparison, the current study showed remarkably higher values, ~0.75–0.92, overlapping similarly across densities. A possible cause of this discrepancy between studies may be that similar R:FR ratios could be achieved at different levels of interception due to a different sowing pattern [a square grid in Evers et al. (2006) compared with row crop in this study], changes in the proportion of stem to leaf and leaf angle (by cultivar and sowing density), leaf thickness, and nutrient content. As a signal to stop tillering, the R:FR threshold seems more consistent across genotypes and environments than intercepted PAR level, in line with its theoretical function of precluding morphogenic responses to density before an important depletion in energy availability.

Figure 7. WSC concentration (mg g⁻¹) in stems at anthesis as a function of stem number m⁻² (a, c), and N concentration (%) in the stems (b, d). In a and b, BLUEs of the variables are standardized relative to the site mean (Materials and methods), including all treatments in trials from 2006–2008 for all genotypes. In c and d, only data from 2008 trials are presented. Data are coloured per treatment within trial to facilitate comparisons. Genotypes are identified by initials (parents) or the three last numbers (RILs).
Casal et al., 1986). In sorghum, shade and defoliation have been shown to act through independent pathways to regulate bud outgrowth (Kebrom et al., 2010).

Finally, high density resulted in increased sheath/internode length (and a lower leaf number, similarly to barley; Kirby and Faris, 1970, 1972). Kirby and Faris (1972) reported that increasing density had no effect on tiller initiation but tillers of higher order failed to elongate. An assay testing genotypic differences in primary root numbers and angle (Manschadi et al., 2008) did not detect any strong link between tillering ability and number of axes of the seminal root system (JTC, personal communication, data not presented).

**Drivers for WSC accumulation: can we separate them from tillering?**

WSCc was negatively related to stem number. This study has shown that management practices resulted in a larger absolute change in maximum and final tiller number per plant or WSCc level than genotypic variation. However, genotypic differences in WSCc were maintained throughout treatments, even under the restrictive environment of the late sowing and, more importantly, when compared at similar stem density (illustrated in Fig. 7). These results together with the evidence on morphological differences per se or brought about by differences in tiller number (see below) point to WSCc being an emergent property of tillering differences with a consistent genotypic component. The complex underlying dynamics between traits could be manipulated to change the balance between number of tillers and WSC accumulation (Fig. 10). From a morphological point of view, this study has shown that the stem wall area of a particular internode was thicker for LT–high WSCc lines than their counterparts. In LT lines, the higher individual leaf area and particular stem morphology could help to establish primary tillers as stronger carbon sinks to the detriment of later/higher order sinks. In addition to morphological changes, the heterogeneity in the population of tillers per plant may also contribute to the low WSCc at lower density or in HT lines. In rice, plants with more stems per plant have a greater dispersion of tiller size, with later tillers achieving lower weight and soluble sugar concentration at anthesis (Mohapatra and Kariali, 2008) and presenting poorer vascularization (Hayashi, 1974). Hence, each new tiller will effectively lower the WSCc at the plant level. Higher biomass partitioning to stem than to leaves resulting from differences in tillering (Dreccer et al., 2009) and a gene expression profile biased towards higher net fructan deposition are likely contributors to high WSCc in the LT lines (Xue et al., 2008), when compared with HT lines at similar stem density. The relationship between these variables is captured in Fig. 10.

Reports of a negative correlation between WSCc and Nc in total biomass (van Herwaarden et al., 1998b; Ruuska et al., 2008) were the motivation to use the approach developed by Greenwood et al. (1991), which describes the changes in Nc as the crop accumulates biomass during the season. Contrary to the expectation of finding different relationships or genotype grouping for lines contrasting in WSCc, a single curve described the decline of Nc with crop ontogeny and biomass for all the genotypes across all trials. This could be expected given the lack of or marginal genotypic differences in total N uptake. The single curve description, however, obscures the internal dynamics of N partitioning in the plant between stems and leaves. Lower stem Nc in LT lines is likely to be linked to a smaller total area of sheaths per plant (this study) or differences in stem C/N metabolism (McIntyre et al., 2011).

**Conclusions and implications for modelling and breeding**

Simulation models are one avenue to investigate the dynamic relationships between tillering and WSC accumulation. Models
that simulate changes in allocation of assimilates between growing organs and incorporate aspects of morphogenesis are EcoMeristem (Luquet et al., 2006), validated for the vegetative phase of rice, and LINGRA (Schapendonk et al., 1998; Rodriguez et al., 1999), developed for swards. Dingkuhn et al. (2006) were able to simulate a low tillering rice cultivar by parameterizing EcoMeristem with a higher seed and leaf 1 weight, lower leaf appearance rate, and a higher carbohydrate threshold for tillering. Neither the sorghum (Kim et al., 2010a; van Oosterom et al., 2011) nor the rice studies reach the stem elongation phase and concomitant sugar storage. However, Dingkuhn et al. (2006) depict the differential accumulation of sugars in sheaths prior to stem elongation in relation to tillering affected by P deficiency. From their results, it would seem possible to simulate increased WSC accumulation as an emergent property of changes in stem number per plant or unit area. Including intraplant variability due to cohorting, stem volume (e.g., functional structural models) or differential metabolic activity would make the simulation of WSCs at the whole-plant level more realistic, allowing for current gene or QTL information on component traits to be used as part of model parameterization. Such a level of detail will be necessary to predict phenotypes ranging in tillering and WSCc, needed for specific adaptation. For instance, lines with a low number of spikes m⁻² and high WSC accumulation have shown superior performance under terminal drought and in irrigated crops in subtropical, short growing seasons (Dreccer et al., 2008; Rattey et al., 2009), but a high spike number is a desirable attribute as water availability increases in cooler and longer growing seasons (Zhang et al., 2010) and high spike number and high WSC accumulation have been key factors behind recent breeding progress in the UK (Shearman et al., 2005).

Fig. 9. Maximum green stem number per plant (y1 from Fig. 1) versus leaf appearance rate (a), final leaf number (b), growth rate per plant (c), and relative growth rate per plant between emergence and DC30 (end of tillering) (d) at normal (08SBTiller-12) and late (08SBTiller-4) sowing in 2008.
The negative relationship between organ number and sugar accumulation is not unique to cereals or constrained to tiller and WSCc storage. It is also ubiquitous in fruit production; Bertin et al. (2010) illustrate it for peach sweetness, while Ferraris and Charles-Edwards (1986b) describe it for grain versus sweet sorghum, and it extends to the much studied grain number–grain weight relationship in wheat. While these negative relationships are consistent with evolutionary considerations (Sadras, 2007), genotypic differences exist within a given range of organ number that are worth selecting for, albeit with a much smaller quantitative effect than the environmental impacts (Sadras and Slafer, 2012). In some cases, pleiotropic effects of a single gene could explain changes in morphology, tillering, and WSCc at the same time. In rice, loss of function of OsTB1 results in the fine culm 1 (fc1) mutant with enhanced tillering and thinner stems (Takeda et al., 2003). Liu et al. (2009) showed that transgenic millet constitutively overexpressing the SiPf40 gene had enhanced tillering, and xylem vessels were enlarged and xylary fibres increased in comparison with the wild type. The gene knock-down restricted tillering, increased phloem differentiation, and reduced xylem for its vascular bundle.

The results of the present study point to complex interactions between development and growth processes over time, resulting in WSCc being an emergent property with a smaller but distinct genotypic component. From a breeding perspective, it is worth highlighting that both parental lines had an intermediate tillering level but Seri had higher WSCc than Babax, a rare combination. Breeders may be able to achieve this by targeting specific component traits with no apparent trade-off (Fig. 10). For instance, a current interest in wheat and barley breeding is to increase grain number and yield potential by stretching the phase of stem elongation without changing the flowering date (Slafer et al., 2001), which effectively means shortening the tillering phase. The present results indicate that, in such a case, a faster phyllochron would compensate for tiller number and, more crucially, spike number. Additionally, increasing the growth rate during stem elongation (e.g. via higher radiation use efficiency) or selecting for a high metabolic rate of WSC deposition could provide

![Fig. 10. Hypothetical relational diagram with the main processes affecting and linking tillering and WSCc at the plant or crop level. An example of a combination leading to high tillering (HT)–low WSCc lines is indicated in parentheses with the direction of change required. For example, a HT line could result from a short phyllochron and a long time to transition to the reproductive stage, leading to a high final leaf number and hence potential tiller number; if senescence is low, this will result in a high final tiller number. More tillers from higher cohorts lower the potential for WSC accumulation (due to smaller diameter and poor vascularization; see the Discussion). The ‘tipping’ arrow scheme between final tiller number and WSCc illustrates their negative relationship: when the stem number increases, WSCc decreases. Genotypic factors such as culm morphology and enzymatic profile also influence WSC accumulation. The daily growth rate responds to temperature and radiation, and influences tillering but also WSC reserves. An R:FR threshold can block further tiller outgrowth.](image-url)
enough reserves to realize the value of a higher grain number (Fig. 10).

Supplementary data

Supplementary data are available at JXB online.

Figure S1. Number of stems per plant versus thermal time from emergence on the normal (a–f) or late (g–l) sowing date.

Figure S2. Fraction of PAR intercepted as a function of thermal time from emergence in 2008, normal sowing.

Table S1. Means and standard error of selected parameters of tillering dynamics curves corresponding to Fig. 2 and Supplementary Fig. S1.

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