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Research

A physiological framework to explain genetic and environmental regulation of tillering in sorghum

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Summary

- Tillering determines the plant size of sorghum (*Sorghum bicolor*) and an understanding of its regulation is important to match genotypes to prevalent growing conditions in target production environments. The aim of this study was to determine the physiological and environmental regulation of variability in tillering among sorghum genotypes, and to develop a framework for this regulation.
- Diverse sorghum genotypes were grown in three experiments with contrasting temperature, radiation and plant density to create variation in tillering. Data on phenology, tillering, and leaf and plant size were collected. A carbohydrate supply/demand (S/D) index that incorporated environmental and genotypic parameters was developed to represent the effects of assimilate availability on tillering. Genotypic differences in tillering not explained by this index were defined as propensity to tiller (PTT) and probably represented hormonal effects.
- Genotypic variation in tillering was associated with differences in leaf width, stem diameter and PTT. The S/D index captured most of the environmental effects on tillering and PTT most of the genotypic effects.
- A framework that captures genetic and environmental regulation of tillering through assimilate availability and PTT was developed, and provides a basis for the development of a model that connects genetic control of tillering to its phenotypic consequences.

Introduction

Tillers are a fundamental component of plant architecture that regulate yield (Kuraparthy *et al.*, 2008) via direct effects on the number of panicles formed (Beall *et al.*, 1991). Like the main shoot, they consist of a succession of phytomers that each contain a leaf blade, leaf sheath and stem internode. Tillers grow from axillary buds located in the axils of leaves, and their development involves the initiation of an axillary meristem, formation of the axillary bud and its subsequent outgrowth (Schmitz & Theres, 2005). These three stages are regulated by the external environment, internal genetic background and their interactions (Beveridge *et al.*, 2003; Shimizu-Sato *et al.*, 2009; Whipple *et al.*, 2011). Each axillary meristem has a window of opportunity for its outgrowth that is associated with the timing of expansion of subtending leaves (Lafarge *et al.*, 2002; Kim *et al.*, 2010b). Tillers also play an important role in biomass accumulation, as intercepted radiation is increased with the greater leaf area associated with tillering. Hence, high-tillering genotypes are better suited to favourable environments where they can maximize resource use. By contrast, in adverse environments, where water for transpiration is limited, low tillering is preferred to restrict plant size and thus increase post-anthesis water availability and grain yield (Hammer, 2006). Under water-limited conditions, excessive tillering can lead to high tiller abortion, poor grain set and small panicle size, thereby reducing grain yield (Kariali & Mohapatra, 2007). To optimize grain yield, it is necessary to have a plant type with the appropriate productive tiller number and plant architecture for the prevailing conditions. Therefore, a clear understanding of the regulation of tillering in cereals is required to enable the matching of the genotypic capacity to produce tillers to the target population of environments in order to achieve high grain yield (Saracutu *et al.*, 2010).

In conditions in which tillering is not affected by water or nitrogen stress, early growth of tillers depends on the availability of excess carbohydrate, which depends on the balance of supply (S) through the photosynthesis of expanded main shoot leaves and demand (D) for growth by the main shoot (Kirby *et al.*, 1985; Lafarge, 2006). The carbohydrate S/D ratio has been used to relate tiller outgrowth to plant internal competition for resources between main stem and tillers in rice (Dingkuhn *et al.*, 2006; Luquet *et al.*, 2006), sorghum (Kim *et al.*, 2010a,b) and wheat (Bos & Neuteboom, 1998). The S/D ratio is a complex indicator of internal plant carbohydrate status, in which solar

radiation and leaf area determine carbohydrate supply via photosynthesis, whereas temperature and leaf area growth determine carbohydrate demand associated with crop development (Hammer *et al.*, 1993; Tardieu *et al.*, 1999; Kim *et al.*, 2010b). Assimilate demand by the main stem increases at high temperatures (Bos & Neuteboom, 1998) in response to an increased rate of leaf area expansion, associated with more rapid leaf appearance (Lafarge *et al.*, 1998). Assimilate supply per plant is reduced by low light interception, which can be a consequence of low incident radiation, short photoperiod, high plant density or defoliation (Bos & Neuteboom, 1998; Gautier *et al.*, 1999). In this context, increased leaf size in early development stages could reduce tillering, as large leaves increase early vigour of the main shoot and hence carbon demand. A negative correlation between tiller number and leaf size has been reported for sorghum (Kim *et al.*, 2010a), perennial ryegrass (*Lolium perenne*) (Bahmani *et al.*, 2000), pearl millet (*Pennisetum glaucum*) (van Oosterom *et al.*, 2001), wheat (*Triticum aestivum*) (Rebetzke & Richards, 1999) and rice (*Oryza sativa*) (Tivet *et al.*, 2001). Although negative correlations between tillering and plant height have been observed in rice (Richards, 1988), the relationships between other leaf and plant size traits and tillering have not been fully elucidated.

Recent studies on tillering in a small number of sorghum genotypes have revealed that, although the S/D balance can explain part of the observed genotypic variation in maximum tiller number, the genotypes also differ in their propensity to tiller (PTT), which represents the endogenous genotypic differences in tillering that cannot be explained by differences in the S/D balance (Kim *et al.*, 2010a). There is some understanding of the endogenous hormonal gene network that controls axillary branching in *Arabidopsis*, tomato, petunia and pea (Dun *et al.*, 2006; Doust, 2007; McSteen, 2009; Yaish *et al.*, 2010). Auxin from the shoot apical meristem inhibits the outgrowth of axillary tiller buds, whereas acropetal movement of cytokinin and strigolactones promotes and inhibits the growth of tiller buds, respectively (Beveridge, 2006; Gomez-Roldan *et al.*, 2008; Ongaro *et al.*, 2008; Umehara *et al.*, 2008; McSteen, 2009). Some orthologous genes of these gene networks have also been found in rice and maize (Li *et al.*, 2003; Takeda *et al.*, 2003; Mao *et al.*, 2007), and these have been found to control shoot branching through the alteration of different transcriptional and hormonal pathways (reviewed in Yaish *et al.*, 2010). In addition, genes have been identified that regulate tillering by integrating extrinsic signals with endogenous cues, such as the *gt1* gene in maize, for which the expression is induced by the shade avoidance response (red : far red (R : FR) ratio of light) and depends on *tb1* (*teosinte branched 1*) activity (Whipple *et al.*, 2011). Genotypic differences in PTT could thus represent differences in the hormones and intrinsic genotypic cues that regulate tillering.

Crop genotypic diversity and the associated growing environment contribute to phenotypic plasticity in tillering. However, the understanding of the physiological and genetic control of tillering in sorghum remains limited, and an improvement in this understanding could have significant implications for plant breeding. Hence, this study aimed: to explore the extent of genetic variability for tillering in sorghum; to determine the physiological and environmental regulation of the genetic variability in tillering; and to develop a framework to explain the environmental and genetic control of tillering. Our study hypothesized that the carbon S/D balance and the intrinsic tillering propensity could explain the physiological and environmental regulation of tillering variability across a wide range of sorghum germplasm.

Materials and Methods

Genetic material

A combination of 51–61 sorghum (*Sorghum bicolor* (L.) Moench) inbred lines and 39 hybrids were grown in three experiments. The inbred lines represented a diverse range of germplasm originating from North America, Australia, Africa and Asia (Table 1). They included parents of mapping populations, and male and female parents of hybrids.

Experimental details

One glasshouse (Expt 1) and two field (Expt 2 and Expt 3) experiments were carried out under contrasting temperature and radiation regimes to generate high and low-tillering conditions. Expt 1 was sown in September 2008 in a glasshouse at the University of Queensland, St Lucia, Qld, Australia (27°28′S, 153°1′E). Expt 2 and Expt 3 were sown in December 2008 and January 2010, respectively, in a field at Hermitage Research Facility, Warwick, Qld, Australia (28°12′S, 152°5′E). In Expt 1, maximum and minimum air temperatures and total radiation were logged daily using a data logger (CR10; Campbell Scientific, Logan, UT, USA). For Expt 2 and Expt 3, weather data were recorded at a centrally located weather station. Average daily radiation, minimum and maximum temperatures, and thermal time were calculated for the first 45 d after emergence, coinciding with the period of tiller appearance. Thermal time was calculated from hourly data, using a broken linear relationship with cardinal temperatures of 11, 30 and 42°C for the base, optimum and maximum temperatures, respectively (Hammer *et al.*, 1993).

All three experiments were laid out as randomized complete block row and column designs with three replications. Genotypes were arranged using neighbour balance to allow for two-dimensional spatial adjustments. Expt 1 included 51 inbred lines and 39 hybrids, and was laid out in six columns of 45 pots each to account for potential temperature gradients in the glasshouse. Pots of 30 cm in diameter were filled with pre-sterilized and pre-fertilized University of California soil mix (containing sand and peat). Four seeds were sown in each pot and, after emergence, plants were gradually thinned to one plant per pot by the four-leaf stage. The whorl of each axis in each plant was sprayed daily with 0.3% $Ca(NO_3)_2$ after establishment to minimize symptoms of calcium deficiency. Three weeks after sowing, a 2% solution of liquid fertilizer aquasol (23% N, 4% P and 18% K in a composition of mono-ammonium phosphate, potassium nitrate,

Table 1 Origin and characteristics of the 51 sorghum (*Sorghum bicolor*) inbred lines used in the experiments

Genotype	Origin	Characteristics
	Africa	
Tx642(B35)	Ethiopia	Partially converted IS 12555, highly stay-green
IS 8525	Ethiopia	Parent of mapping population for ergot resistance
SC111-14E	Ethiopia	Fully converted zera zera landrace Gambela No 6
SC103-14E	Ethiopia	Conversion of a breeding line from South Africa, guinea-caudatum
SC108C	Ethiopia	Fully converted bicolor-kafir landrace Gambela No 1
SC170-6-8	Ethiopia	High yielding, partly converted version of IS12661 a caudatum line ex Ethiopia
SC23	Ethiopia	A durra genotype
SC35C	Ethiopia	Fully converted durra landrace IS 12555, source of stay-green
SC999	Ethiopia	Partially converted durra-bicolor landrace IS 11080
ISCV400	Mali	Bred by ICRISAT as a food sorghum in Mali, 2-dwarf, white grain
Malisor 84-7	Mali	Advanced line from Mali, described as kafir-caudatum
SC1075-8	Nigeria	Partial conversion of a landrace from Nigeria
MP531	Southern Africa	Breeding line, 2-dwarf, obtained via TAMU
SC56-14E	Sudan	Source of stay-green drought resistance, fully converted caudatum landrace
SC62C	Sudan	A high-tillering genotype, fully converted caudatum-bicolor landrace
SC636-6	Uganda	Partial conversion of a caudatum landrace
	America	
Dorado	El Salvador	Moderately fungal disease resistant, hard endosperm
Karper 669	USA	Diverse yellow endosperm germplasm line
KS115	USA	Large seed
MLT135	USA	Elite moderately senescent parent line ex TAMU breeding programme
R9188	USA	Partially converted derivatives of sweet sorghum Rio
R9733	USA	Breeding line from Texas A&M University breeding programme
Rio	USA	Sweet sorghum
TAM422	USA	Early hybrid parent lacking in stay-green drought resistance
Tx2536	USA	Early hybrid parent lacking in stay-green drought resistance
Tx2737	USA	High-yielding, yellow endosperm, widely used as parent commercially in the USA
Tx2895	USA	Widely used commercially in the USA
Tx430	USA	Yellow endosperm, widely used as parent commercially in the USA
TX623	USA	An elite US female pedigree BTx3197/SC170-6-4-4
TX7000	USA	Early hybrid parent lacking in stay-green drought resistance
	Asia	
Ai4	China	2-dwarf, photoperiod-insensitive, possible cold tolerance
LR2490-3	China	Breeding line, 2-dwarf, classified as zera zera
LR9198	China	Breeding line, 2-dwarf, male of a good hybrid in China
ICSV745	India	Parent of mapping population
M35-1	India	Drought resistant
RS29	India	Drought resistant
	Australia	
B923296	Australia	Elite stay-green parent ex QPIF breeding programme
QL12	Australia	Source of stay-green drought resistance
QL33	Australia	Elite moderately senescent parent line ex QPIF breeding programme
QL36	Australia	Elite moderately senescent parent line ex QPIF breeding programme
R890562	Australia	Elite moderately senescent parent line ex QPIF breeding programme
R931945-2-2	Australia	Elite stay-green parent ex QPIF breeding me
R9403463-2-1	Australia	Elite moderately senescent parent line ex QPIF breeding programme
R993396	Australia	Elite moderately senescent parent line ex QPIF breeding programme
R999003	Australia	Selected from an interspecific cross between *S. arundinaceum* (African wild-type high tillering) and R931945-2-2 (low tillering)
R999017	Australia	As for R990003
R999066	Australia	As for R990003
R999081	Australia	As for R990003
R999110	Australia	As for R990003
R999197	Australia	As for R990003
R999218	Australia	As for R990003

urea, potassium chloride, zinc sulfate, copper sulfate, sodium molybdate, manganese sulfate, sodium ferric EDTA and sodium borate) was added in two consecutive weeks to provide additional nitrogen and to ensure that nutrients were non-limiting. Watering was performed regularly and no drought stress occurred. The experiment was terminated after the completion of flowering.

The field experiments (Expts 2 and 3) included the 90 genotypes of Expt 1 and an additional 10 Near Isogenic Lines (NILs). Plots were distributed in 30 ranges of 10 rows and consisted of one row of 4.75 m in length each. The sites were fertilized and cultivated before planting. The experiments were machine planted with a row spacing of 75 cm and thinned at the three-leaf stage to a plant-to-plant spacing of >70 cm (Expt 2) or 45–50 cm (Expt 3). Weeding was performed as and when necessary. Experiments were rain-fed and terminated after the completion of flowering.

Table 2 Abbreviations of plant parameters used in this study

Abbreviation	Trait
DTA	Days to anthesis (d)
ID	Internode diameter (cm)
IL	Inflorescence length (cm)
LA5	Area of Leaf 5 (cm^2)
LL	Length (cm) of designated leaf number
LLIR	Leaf length increase rate from 5th to 9th leaf (cm per leaf)
LW	Width (cm) of designated leaf number
LWIR	Leaf width increase rate from 5th to 9th leaf (cm per leaf)
N_BT	Basal tiller number
N_FT	Fertile tiller number
N_TT	Total tiller number
PH_BFL	Plant height from base to flag leaf (cm)
PH_BI	Plant height from base to joint of inflorescence (cm)
Phyl	Phyllochron (°Cd per leaf)
PTT	Propensity to tiller
S/D	Supply/demand index
TLN	Total leaf number
TPH	Plant height from base to inflorescence apex (cm)

Observations

Data on leaf and plant size, leaf appearance and tiller number were recorded on one plant per genotype in each replication in each experiment. The number of visible and fully expanded leaves on the main shoot and the number of emerged tillers were recorded three times a week. A leaf was considered to be visible if its tip was visible inside the whorl, and fully expanded if its ligule was visible above the ligule of the previous leaf. Abbreviations of measurements taken on different plant parameters are shown in Table 2. Total leaf number (TLN) at anthesis was the number of fully expanded leaves produced on the main shoot. Leaf appearance rate (LAR) was calculated as the slope of the regression of fully expanded leaf number on cumulative thermal time, excluding the last few leaves that appear at a faster rate. Leaf size was represented by the final leaf length (LL) and maximum leaf width (LW) of main shoot leaves 5, 7 and 9. Leaf area (LA) was obtained by multiplying the length and width by a shape coefficient of 0.69 (Kim *et al.*, 2010b). The internode diameter (ID) was measured using digital slide callipers on the narrowest region of the first internode above the basal root zone, after removal of the leaf sheath. Plant height was measured from the base of the plant to the flag leaf (PH_BFL) or the base of the inflorescence (PH_BI). Adding the measurement of the inflorescence length (IL) yielded the total plant height (TPH). Days to anthesis (DTA) was measured as the number of days after emergence to the first date of pollen shed. Tillers were named after the main shoot leaf axil from which they appeared; for example, T3 appeared from the axil of Leaf 3. Basal tiller number (N_BT) included only primary tillers, whereas total tiller number (N_TT) included primary, secondary and tertiary tillers. Fertile tiller number (N_FT) included all tillers that produced a panicle.

A plant carbohydrate S/D index was estimated to quantify environmental and genotypic effects on tillering using a modified version of the index presented by Kim *et al.* (2010a):

$$\text{S/D index} = \frac{\text{RAD}_{\text{LED5}} \times \text{LA}_{\text{L5}} \times \text{phyllochron(ligule to ligule)}}{\text{LLIR}(5-9) \times \text{LWIR}(5-9)} \quad \text{Eqn 1}$$

where RAD_{LED5} is the average incident global radiation per unit thermal time (MJ m^{-2}°Cd^{-1}) during the period of expansion of main shoot leaf 5 (LED5, °Cd), LA_{L5} is the fully expanded area of L5, which was expanding at the start of tillering, and LLIR (5–9) and LWIR(5–9) are the linear rates of increase in the maximum leaf length and maximum leaf width, respectively, for successive leaves between L5 and L9. The duration of expansion of main shoot leaf 5 (LED5) was calculated as the thermal time between the appearance of its tip and ligule. The phyllochron (Phyl, °Cd per leaf), which is the average thermal time for appearance per leaf from Leaf 5 to Leaf 9, was used in this study in place of LED5 which was employed by Kim *et al.* (2010a). The phyllochron does not require observations on leaf tip appearance and, because it is the average appearance across five leaves, its value is likely to be more robust than LED5 used by Kim *et al.* (2010a). The numerator of Eqn 1 is considered as an index of carbohydrate supply to the plant during tillering and the denominator as an index of carbohydrate demand by the main shoot. Hence, a high S/D index would favour tillering. The S/D index was calculated for each genotype in each experiment, using the average of the S/D indices calculated for individual replications.

The PTT of each genotype was calculated from the relationship of N_TT to the S/D index. Initially, a linear regression of N_TT on the S/D index was fitted across data from all genotypes and experiments (excluding SC62C germplasm, which produced secondary tillers). The average PTT was derived as the *y*-intercept of the regression (i.e. N_TT when the S/D index is zero). The vertical deviation of each point from the regression line was used to adjust the average PTT to obtain the PTT estimate for each genotype in each experiment. A high value for PTT indicated an above average tiller number for a given S/D index. The average PTT across the three experiments for each genotype was taken as the PTT for that genotype.

Data analyses

Statistical analyses were performed using a linear mixed model for each trait. Variance estimates of genetic parameters and genotypic values of each trait were derived from a REML mixed model allowing for spatial variation within each experiment (Gilmour *et al.*, 1997). The general form of the mixed model is:

$$\mathbf{y} = \mathbf{X}\tau + \mathbf{Z}_g\mathbf{u}_g + \mathbf{Z}_0\mathbf{u}_0 + \mathbf{e} \quad \text{Eqn 2}$$

where the vector $\mathbf{y}$ represents the observed values of a trait with length n, $n = i \times j \times k$ with i experiments ($i = 3$), j genotypes ($j = 100$) and k replicates ($k = 3$). $\mathbf{X}$ is the design matrix for the fixed effects and $\boldsymbol{\tau}$ is the vector of fixed effects containing a mean trait value for each experiment. $\mathbf{Z}_g$ is the design matrix for the random genetic effects and $\mathbf{u}_g$ represents the $i \times j$ random effects for genotypes in environments. $\mathbf{Z}_o$ is the design matrix for the peripheral random effects and $\mathbf{u}_o$ represents the random effects

for the peripheral terms. The peripheral terms include replication and effects caused by rows and columns for each environment, and a within-plot effect for Expt 1.

The genotype effects $\mathbf{u}_g$ have a normal distribution with zero mean and variance given by $\mathbf{I} \otimes \mathbf{G}$, where $\mathbf{I}$ is a $(j \times j)$ matrix containing '1' on the diagonal and '0' elsewhere and $\mathbf{G}$ is a $(i \times i)$ matrix that controls the structure of the genotype by environment interaction. In across-experiment analyses, correlated error terms in the variance–covariance matrix structure $\mathbf{G}$ are required to allow for the heterogeneity of error variance across environments (Borràs *et al.*, 2009). $\mathbf{G}$ is either a diagonal matrix with genetic variances on the diagonal and '0' on the off-diagonals, analogous to a single-site analysis, or a correlation matrix containing genetic variances on the diagonal and between site co-variances on the off-diagonals. Genotype × environment (G × E) interaction is assessed by examination of the correlation $\mathbf{G}$ matrix.

An initial assessment of the significance of G × E interactions in a mixed model of the form of Eqn 2 was modified to allow fixed effects for genotypes, experiments and their interaction with random effects as before, including all the extraneous errors ($\mathbf{u}_0$). This model was used to provide a statistical test in the form of a Wald test to test for a significant G × E interaction. Predicted phenotypic values for each genotype in each experiment were estimated from best linear unbiased predictors (BLUPs) from the random effects model. Both the fixed and random versions of the models were fitted using REML linear mixed procedures in Genstat 13.0 (Payne *et al.*, 2009). Estimates of variance components for genetic variance and error variance (σ^2_g and σ^2_e) were used for the estimation of broad-sense heritability and coefficients of variation within each site.

To indicate the magnitude of variability, coefficients of variation were estimated using:

$$\text{Phenotypic coefficient of variation, PCV} = \frac{\sqrt{\sigma^2_{ph}}}{\bar{X}} \times 100 \quad \text{Eqn 3}$$

$$\text{Genotypic coefficient of variation, GCV} = \frac{\sqrt{\sigma^2_{g}}}{\bar{X}} \times 100 \quad \text{Eqn 4}$$

where σ^2_{ph} is the phenotypic variance, σ^2_g is the genotypic variance and $\bar{X}$ is the grand mean. Although GCV indicates the extent of genetic variation of a trait, this is insufficient to describe the perpetuation of genes from one generation to the next (Johnson *et al.*, 1963). Therefore, broad-sense heritabilities were estimated to indicate the effectiveness of selection of genotypes based on phenotypic performance. As a result of a mixed model containing random spatial effects, the general formula for broad-sense heritability does not apply; instead, an approximation of broad-sense heritability can be calculated for each experiment using the formula of Cullis *et al.* (2006):

$$\text{Broad-sense heritability } H = 1 - \frac{avSED^2}{2\sigma^2_g} \quad \text{Eqn 5}$$

where $avSED^2$ is the average pairwise prediction error and σ^2_g is the within-site genetic variance components resulting from the mixed model analysis.

The estimation of genetic correlation among traits and principal component analyses were performed in Genstat 13.0 (Payne *et al.*, 2009) using BLUPs. Biplots were used to approximate the genetic correlation between two variables from the cosine angle formed by their vectors, only when the first two principal components represented most of the variation. When the two principal components failed to explain most of the variation, genetic correlations were derived from direct estimation (Borràs *et al.*, 2009). As the 10 NILs were not included in all three experiments, standardized values of these entries were excluded from results other than variance analyses.

Results

Environmental and genotypic variation in tillering

Average radiation in the glasshouse experiment (Expt 1) was 2.5–3-fold lower than that in the field experiments (Expt 2 and Expt 3), but the average temperature was 3.5–4°C higher (Table 3). In the field experiments, Expt 2 received less rainfall than Expt 3, and this was combined with higher average daily radiation and maximum temperature. The genotypic means for total tiller number, estimated from single-site analyses as best linear unbiased estimators (BLUEs), were consistently lower in Expt 1 than in Expts 2 and 3 (Fig. 1; Supporting Information Tables S1, S2), indicating the negative effects of low radiation, high temperature and high plant density on tillering in the glasshouse experiment.

The variation in total and fertile tiller number was narrower for hybrids than for inbred lines (Tables 4, 5) and, on average, the CV for N_FT and N_TT for inbred lines was nearly double that for hybrids. In all three experiments, SC62C produced the highest tiller number among the inbred lines and in hybrid form, as it was the only genotype that produced secondary and tertiary tillers. In inbred lines, total tiller number varied from 0.0 to 9.5 (Expt 1), 0.6 to 15.6 (Expt 2) and 0.9 to 12.7 (Expt 3), whereas, for fertile tillers, it ranged from 0 to 5.6 (Expt 1), 0.3 to 13.7 (Expt 2) and 0.7 to 11.9 (Expt 3). Variance components differed significantly within and across experiments (Table 6).

The initial assessment using fixed genotype and experiment effects gave a highly significant G × E interaction for total tiller number (Table 6, Fig. 1). However, the statistically more accurate model for these data was the random effects genotype by experiment with a common correlation model, where each experiment has a different genetic variance. The correlation between experiments was determined to be 0.917, indicating a high agreement on genetic ranking between the experiments. The estimate of broad-sense heritability of this trait was high within all experiments (Table 6). The higher broad-sense heritability in Expt 2 compared with the other two experiments was associated with the increased σ^2_g in that experiment.

Table 3 Attributes and environmental conditions for the three experiments

Environmental parameters	Expt 1	Expt 2	Expt 3
Experimental sites	St Lucia, glasshouse	Warwick, field	Warwick, field
Sowing date	11 September 2008	17 December 2008	3 February 2010
Spacing	30 cm × 30 cm	75 cm × 75 cm	50 cm × 75 cm
Average radiation[a] (MJ m^{-2} d^{-1})	7.8	24.9	18.3
Average daily minimum temperature[a] (°C)	20.2	16.0	16.7
Average daily maximum temperature[a] (°C)	31.9	28.7	26.0
Thermal time (°C d^{-1})	13.1	10.6	10.0
Total rainfall[b] (mm)	Irrigated	40.9	160.3

[a]For the period up to final primary tiller appearance.
[b]For the period until anthesis.

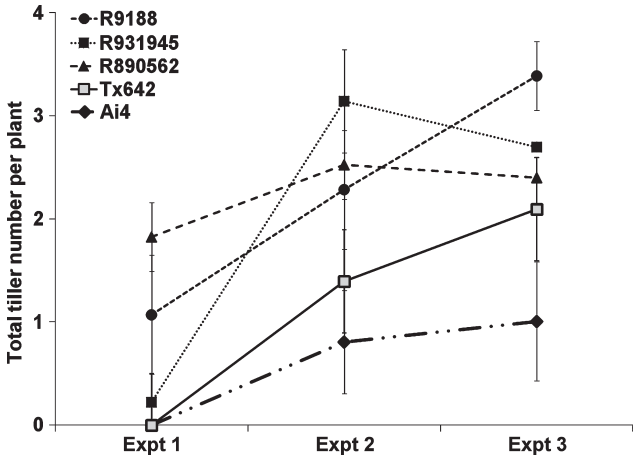

Fig. 1 Total tiller number per plant for five selected sorghum (*Sorghum bicolor*) genotypes in the three experiments conducted in this study. Error bars show ± 1SE.

The PCV was consistently greater than the GCV (Table 6), indicating that the observed variation in total tiller number was caused by both genotypic and environmental effects. Variation in GCV and PCV across experiments indicated that genotypic variation in tillering was environment dependent. The combination of high GCV and high broad-sense heritability indicates high genetic regulation of this trait.

Although genetic correlations for total tiller number between pairs of environments ranged from 0.92 (hybrids) to 0.95 (inbred lines in Expt 2 vs Expt 3, Table S3), the patterns of response across the three experiments indicated the existence of some G × E interactions (Fig. 1). In particular, genotypes with different tiller numbers in the high-tillering conditions of Expt 2 and Expt 3 had similar tiller numbers in Expt 1, where many genotypes produced virtually no tillers in its low-tillering conditions. In general, G × E interactions were of a non-crossover type, although some crossover-type interactions occurred for selected G × E combinations (Fig. 1).

Relationship of morphological traits with tillering

Tiller number was only weakly negatively correlated with phenology. Early genotypes that produced few leaves tended to have greater tiller numbers than later genotypes that produced more leaves (Table 7). This weak negative correlation was consistent with the right angle of the respective vectors in the biplot (Fig. 2). Although genetic correlations were stronger (Table 8), it was unlikely that genotypic differences in phenology had a major effect on differences in tillering.

Tiller number was significantly negatively correlated with ID and LWIR in both inbred lines and hybrids (Table 7), consistent with the wide angle between the directional vectors in the covariate-effect biplots (Fig. 2). However, the association between ID and LWIR was weak (Table 7, Fig. 2). The genetic correlation for LWIR and, in particular, ID between pairs of experiments was as high as for tillering itself (Table S3), indicating a robustness of these traits across experiments. LLIR, however, showed no association with tiller number, which may have been a result of the low broad-sense heritability (Table S3) and limited genotypic differences (short arrow in Fig. 2). The correlation of tillering with leaf size (LWIR) and ID suggests that tillering may at least partly be associated with the size of the main shoot, and hence potentially with the S/D index.

S/D index and PTT

The S/D index explained 56% of the variation in total tiller number across genotypes and experiments (Fig. 3). This high R^2 was associated, to a large extent, with environmental effects on tillering across experiments, as the glasshouse experiment (Expt 1) combined a low S/D index with low tiller number. However, the association between S/D index and tiller number was weak (Table 7) and the genetic correlation non-significant (Table 8). Nonetheless, in accordance with Eqn 1, the genetic correlation of S/D with LWIR was significantly negative and that with the area of Leaf 5 (LA5) significantly positive (Table 8). By contrast, the PTT explained 71% of the genotypic variation in tillering (Fig. 4). Like tiller number, the genotypic correlations of PTT with LWIR and ID were significantly negative. Genetic correlations between pairs of experiments and the broad-sense heritability were low for the S/D index, but high for PTT (Table S3). Because the genetic correlation between PTT and tiller number was highly significant (Table 8), the results suggest that environmental differences in tillering were predominantly associated with differences in the S/D index, whereas genotypic differences in tillering were rather associated with intrinsic genotypic differences in an ability to produce tillers.

Table 4 Minimum (MIN), maximum (MAX), mean and coefficient of variation (CV) of observed data for all traits across the three experiments for sorghum (*Sorghum bicolor*) inbred lines

	Expt 1				Expt 2				Expt 3			
Traits[a]	MIN	MAX	Mean	CV	MIN	MAX	Mean	CV	MIN	MAX	Mean	CV
TLN	10.4	17.7	14.8	0.11	14.1	22.2	17.8	0.08	12.5	18.7	16.3	0.07
TPH	66.0	293.2	123.3	0.31	68.7	249.4	102.2	0.27	67.0	252.8	110.4	0.29
ID	9.8	21.4	16.1	0.13	12.9	27.0	20.1	0.11	12.4	24.3	18.7	0.12
LA5	14.5	49.7	31.8	0.27	9.7	29.3	19.5	0.24	20.4	52.7	33.5	0.25
Phyl	46.5	60.0	50.7	0.06	33.7	43.1	37.5	0.06	33.5	44.1	38.4	0.07
LLIR	6.22	11.02	9.05	0.12	4.27	8.76	6.07	0.13	5.11	8.93	6.68	0.12
LWIR	0.68	1.43	1.08	0.13	0.66	1.34	0.93	0.15	0.74	1.48	1.1	0.13
S/D	40.9	258.2	128.6	0.37	109.7	588.9	308.2	0.30	167.4	549.2	315.6	0.24
DTA	52.8	72.8	64.7	0.07	55.9	76.1	66.7	0.06	60.5	76.3	69.0	0.06
N_FT	0	5.57	0.14	5.71	0.33	13.66	2.52	0.71	0.7	11.89	2.25	0.69
N_TT	0	9.46	1.11	1.27	0.63	15.6	2.87	0.72	0.85	12.68	2.92	0.56
PTT	−1.11	3.86	0.43	2.49	−0.3	5.18	1.00	0.94	−0.51	5.09	1.14	0.85

[a]For abbreviations of traits, see Table 2.

Table 5 Minimum (MIN), maximum (MAX), mean and coefficient of variation (CV) of observed data for all traits across the three experiments for sorghum (*Sorghum bicolor*) hybrids

	Expt 1				Expt 2				Expt 3			
Traits[a]	MIN	MAX	Mean	CV	MIN	MAX	Mean	CV	MIN	MAX	Mean	CV
TLN	11.0	17.8	14.1	0.11	14.3	23.8	17.1	0.09	13.1	19.0	15.7	0.08
TPH	111.5	158.8	133.3	0.08	91.6	123.9	107.8	0.07	99.5	143.7	121.7	0.09
ID	12.5	21.9	17.1	0.11	15.6	26.8	21.0	0.09	15.7	23.5	19.9	0.08
LA5	18.8	53.8	35.9	0.21	14.0	36.7	21.6	0.21	26.0	51.1	36.6	0.16
Phyl	45.5	55.8	49.7	0.04	32.6	40.2	36.4	0.05	33.3	41.2	37.5	0.04
LLIR	6.22	10.39	8.96	0.10	4.74	7.6	6.31	0.08	4.85	7.7	6.83	0.08
LWIR	0.82	1.41	1.16	0.10	0.75	1.26	1.03	0.10	0.92	1.44	1.17	0.09
S/D	41.4	221.6	120.4	0.32	182.9	391.4	267.7	0.17	221.8	430.8	302.3	0.16
DTA	53.0	68.6	60.8	0.06	55.7	75.3	62.3	0.06	59.9	72.0	64.8	0.04
N_FT	0	2.16	0.2	2.80	1.68	5.4	2.65	0.44	1.71	4.81	2.51	0.29
N_TT	0.05	4.01	1.07	0.90	1.87	7.3	2.87	0.45	1.87	6.68	3.12	0.32
PTT	−0.4	2.8	0.55	1.44	−0.12	3.65	1.3	0.61	0	3.33	1.45	0.48

[a]For abbreviations of traits, see Table 2.

Discussion

This study examined the variability of tillering in a diverse range of germplasm to identify physiological determinants of genotypic differences in tillering. Small leaves and narrow stems were associated with a high tiller number, indicating a role of the carbon S/D index in tillering. This index explained most of the environmental effects on tillering, allowing subsequent identification of causes for genotypic differences in tillering across environments. The S/D index explained some of the genotypic differences in tillering, whereas the PTT, which represents effects that are independent of the S/D index and are probably associated with hormones, explained a major part of these genotypic differences. The results validate the framework developed by Kim *et al.* (2010a,b) across a wide range of sorghum germplasm, and allow the production of a generic tillering framework that provides a basis for the development of a model for the genetic control of tillering.

S/D index captured environmental effects on tillering

The S/D index captured most of the environmental effects on tillering and explained 56% of the variation in the total tiller number across genotypes and experiments (Fig. 3). Each tiller has a window of opportunity for its appearance equivalent to one phyllochron (Kim *et al.*, 2010b). The duration of this period is determined by temperature, but whether this tiller actually grows is partly determined by assimilate availability, which is determined by the radiation intercepted by the plant (Hammer *et al.*, 2010). The low tiller number in Expt 1 relative to Expt 2 and Expt 3 (Fig. 1) was consistent with this theory and with the results of Kim *et al.* (2010a,b). Hence, radiation per unit thermal time during the period of expansion of main shoot Leaf 5 (RED_{LED5}), which is part of the calculation of the S/D index (Eqn 1), provides a powerful means to capture environmental effects on tillering.

Small main shoot organ size is associated with increased tillering

The importance of the carbon status of the crop to tillering was highlighted by the significant association between tillering and LWIR (Tables 7, 8). Small leaves reduce carbohydrate demand by the main shoot and could thus increase carbohydrate availability for tillering (Eqn 1). A negative association between leaf size and tillering has been observed for rice (Tivet *et al.*, 2001), wheat (Rebetzke *et al.*, 2004), sorghum (Lafarge *et al.*, 2002; van Oosterom *et al.*, 2011) and pearl millet (van Oosterom *et al.*, 2001), but also across species, as the decline in leaf width from maize to sorghum to pearl millet is associated with an increase in tillering across these species. Genotypic differences in leaf width are not associated with meristem size *per se*, as environmental stresses that affect leaf width do not necessarily affect apical dome size at leaf initiation (Mitchell & Soper, 1958; Beemster & Masle, 1996). Rather, leaf width is related to the circumference of the subapical meristematic zone of the stem in wheat and *Lolium perenne*, which is related to the cell number along the intercalary meristem (Mitchell & Soper, 1958; Beemster & Masle, 1996). As these cells are generated at the primordial and post-primordial stages (Beemster & Masle, 1996), this increased cell number would lend support to the view that increased leaf width would increase carbon demand by the main shoot.

The negative relationship between tillering and internode diameter (Tables 7, 8) is consistent with the carbon S/D framework that large main shoots are associated with low tillering. Similar results were observed by Borrell *et al.* (2000a,b). Moreover, the main shoot stem diameter of sorghum decreases with increasing plant density (Caravetta *et al.*, 1990), consistent with reduced assimilate availability in response to lower radiation interception per plant. However, the final internode diameter at the base of the stem is only expressed after completion of tillering, making it unlikely that the negative association between internode diameter and tillering is causal. Although it is possible that tillering could drive internode diameter, we hypothesize that the association between the two traits is an emergent consequence of common underpinning processes that determine the expression of both traits, as maximum internode diameter could already be genetically determined in the meristem during tillering. Stem diameter depends on radial growth through lateral meristems (cambium) that are under hormonal control (Ursache *et al.*, 2013). One hormone that promotes cell division in the cambium of dicotyledonous species is strigolactone, which also suppresses tillering (Foo & Reid, 2013). In such a scenario, the negative genetic correlation between tillering and internode diameter (Table 8) could represent common hormonal control. This would support a close association between the carbon (sugar) and hormonal control of tillering.

Table 6 Analyses of variance and estimates of genetic parameters for total tiller number in sorghum (*Sorghum bicolor*) estimated in the three experiments

	df	Sum of squares	Wald statistic	Pr (χ^2)
(Intercept)	1	2378.8	2378.8	<2.2e-16 ***
Site	2	75.52	75.52	<2.2e-16 ***
Genotype	99	2965.87	2965.87	<2.2e-16 ***
Site : genotype	187	512	512	<2.2e-16 ***
Residual (MS)		1		

Genetic parameters	Expt 1	Expt 2	Expt 3
Mean	1.10	2.79	3.03
σ^2_g(SE)	1.15 (0.19)	3.13 (0.48)	1.71 (0.27)
σ^2_e(SE)	0.34 (0.05)	0.52 (0.06)	0.44 (0.05)
Broad-sense heritability	0.91	0.94	0.93
GCV (%)	97	63	43
PCV (%)	110	68	48

***, $P<0.001$; σ^2_g, genotypic variance component; σ^2_e, residual variance component.
PCV, Phenotypic coefficient of variation; GCV, Genotypic coefficient of variation.

Table 7 Phenotypic correlations among the morphological traits of sorghum (*Sorghum bicolor*) inbred lines (above diagonal) and hybrids (below diagonal) across three different environments

Traits[a]	TLN	TPH	ID	LA5	Phyl	LLIR	LWIR	S/D	DTA	N_FT	N_TT	PTT
TLN		0.26^{ns}	0.58***	-0.01^{ns}	−0.61***	-0.01^{ns}	0.15^{ns}	-0.15^{ns}	0.82***	−0.39**	−0.31*	0.11^{ns}
TPH	0.19^{ns}		-0.15^{ns}	0.09^{ns}	-0.03^{ns}	0.11^{ns}	0.19^{ns}	-0.07^{ns}	0.31*	0.00^{ns}	0.00^{ns}	-0.02^{ns}
ID	0.86***	0.04^{ns}		0.31*	−0.28*	0.14^{ns}	0.19^{ns}	0.00^{ns}	0.53***	−0.48***	−0.46***	-0.13^{ns}
LA5	−0.47***	0.27^{ns}	−0.31*		0.04^{ns}	0.06^{ns}	0.28*	0.60***	0.00^{ns}	-0.15^{ns}	-0.12^{ns}	-0.23^{ns}
Phyl	−0.67***	0.02^{ns}	−0.56***	0.52***		0.40**	0.21^{ns}	-0.09^{ns}	-0.21^{ns}	0.21^{ns}	0.13^{ns}	−0.31*
LLIR	−0.66***	0.09^{ns}	−0.68***	0.39**	0.68***		0.25^{ns}	−0.37**	0.20^{ns}	-0.10^{ns}	-0.08^{ns}	-0.24^{ns}
LWIR	0.02^{ns}	-0.09^{ns}	0.17^{ns}	0.41**	-0.10^{ns}	0.00^{ns}		−0.42**	0.24^{ns}	−0.53***	−0.58***	−0.44***
S/D	-0.02^{ns}	0.06^{ns}	0.09^{ns}	0.47***	0.02^{ns}	−0.32*	0.00^{ns}		-0.17^{ns}	0.22^{ns}	0.27^{ns}	0.23^{ns}
DTA	0.92***	0.20^{ns}	0.82***	−0.29*	−0.54***	−0.63***	0.12^{ns}	0.13^{ns}		−0.37**	−0.30*	0.01^{ns}
N_FT	-0.18^{ns}	-0.01^{ns}	−0.32*	-0.18^{ns}	-0.10^{ns}	0.08^{ns}	−0.43**	-0.05^{ns}	-0.24^{ns}		0.96***	0.74***
N_TT	−0.30*	0.16^{ns}	−0.42**	-0.01^{ns}	0.04^{ns}	0.19^{ns}	−0.45**	-0.03^{ns}	−0.35*	0.93***		0.88***
PTT	0.07	-0.18^{ns}	0.01^{ns}	-0.20^{ns}	−0.44***	-0.26^{ns}	−0.37**	0.28*	-0.04^{ns}	0.78***	0.91***	

[a]For abbreviations of traits, see Table 2.
***, $P<0.001$; **, $P<0.01$; *, $P<0.05$; ns, non-significant.

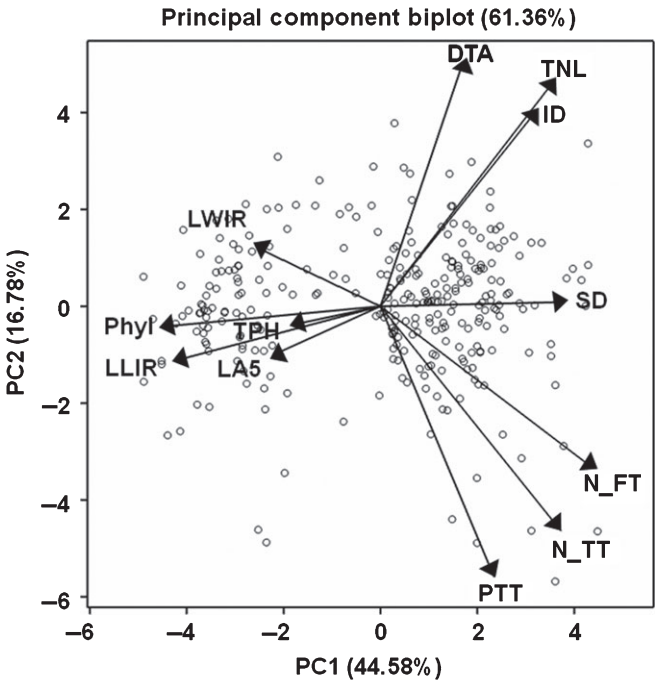

Fig. 2 Principal component (PC) biplot based on the correlation matrix showing genetic correlation among traits of sorghum (*Sorghum bicolor*) across environments. The biplot was constructed from the predicted genotypic values estimated from best linear unbiased predictors (BLUPs) for 85 genotypes (excluding SC62C and its hybrids and Near Isogenic Lines (NILs) of Expt 2 and Expt 3) by reducing extraneous errors of row, column and replication. DTA, days to anthesis; ID, internode diameter; LA5, area of Leaf number 5; LLIR, leaf length increase rate; LWIR, leaf width increase rate; N_FT, fertile tiller number; N_TT, total tiller number; Phyl, phyllochron; PTT, propensity to tiller; SD, supply/demand index; TNL, total number of leaves; TPH, total plant height.

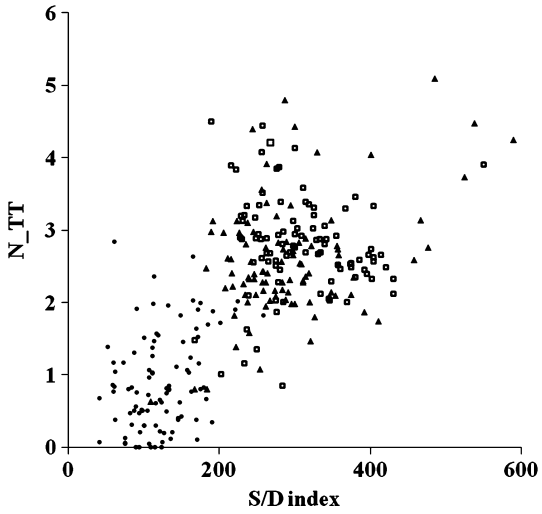

Fig. 3 Total tiller number per plant (N_TT) vs carbon supply/demand index (S/D index) of sorghum (*Sorghum bicolor*) across three experiments (Expt 1, closed circles; Expt 2, closed triangles; Expt 3, open squares). Trait values of 85 genotypes (excluding SC62C and its hybrids and Near Isogenic Lines (NILs)) were estimated from best linear unbiased estimators (BLUEs). Regression of N_TT on the S/D index in these environments was $y = 0.0083x + 0.17$, $R^2 = 0.56$, $n = 255$.

PTT was a major cause of genotypic differences in tillering

The PTT explained > 70% of the genotypic variation in tillering. Genotypes with high PTT have a low threshold S/D index at which tillers start to appear. Such variation in the threshold S/D index supports the involvement of hormonal regulation in the tillering of sorghum. Auxin, cytokinin and strigolactones can each affect tillering, partly through an effect on apical dominance (Beveridge, 2006; Gomez-Roldan *et al.*, 2008; Ongaro *et al.*, 2008; Umehara *et al.*, 2008; McSteen, 2009). Several genomic regions associated with these hormonal pathways have been identified (Lincoln *et al.*, 1999; Snowden *et al.*, 2005; Xu *et al.*, 2005; Beveridge, 2006; Gomez-Roldan *et al.*, 2008; Ongaro & Leyser, 2008; Umehara *et al.*, 2008; McSteen, 2009). Hence, genotypic variation in PTT could be regulated by the intrinsic genetic makeup of sorghum plants.

Although the carbon S/D balance and hormonal control represent distinct physiological processes for the control of tillering, these processes can potentially be coordinated. In sorghum, the content of strigolactone 5-deoxystrigol in the roots is enhanced by low nitrogen and phosphorus availability in the soil

Table 8 Genetic correlations among the morphological traits of sorghum (*Sorghum bicolor*) genotypes (both inbred and hybrids) estimated from three different experiments

Traits[a]	TPH	ID	LA5	Phyl	LLIR	LWIR	S/D	DTA	N_FT	N_TT	PTT
TLN	0.19^{ns}	0.59^{***}	-0.23^{*}	-0.48^{***}	-0.22^{*}	0.00^{ns}	-0.13^{ns}	0.82^{***}	-0.40^{***}	-0.35^{***}	-0.26^{*}
TPH		-0.03^{ns}	0.14^{ns}	-0.10^{ns}	0.10^{ns}	0.19^{ns}	-0.10^{ns}	0.13^{ns}	0.01^{ns}	0.02^{ns}	0.09^{ns}
ID			0.16^{ns}	-0.39^{***}	-0.11^{ns}	0.25^{*}	-0.06^{ns}	0.37^{***}	-0.47^{***}	-0.47^{***}	-0.42^{***}
LA5				0.09^{ns}	0.12^{ns}	0.41^{***}	0.52^{***}	-0.20^{ns}	-0.10^{ns}	-0.06^{ns}	-0.22^{*}
Phyl					0.34^{***}	0.04^{ns}	0.12^{ns}	-0.10^{ns}	0.25^{*}	0.18^{ns}	-0.01^{ns}
LLIR						0.21^{*}	-0.34^{***}	-0.07^{ns}	0.01^{ns}	0.03^{ns}	0.13^{ns}
LWIR							-0.36^{***}	-0.02^{ns}	-0.45^{***}	-0.51^{***}	-0.41^{***}
S/D								-0.03^{ns}	0.14^{ns}	0.19^{ns}	-0.14^{ns}
DTA									-0.37^{***}	-0.32^{***}	-0.31^{**}
N_FT										0.95^{***}	0.82^{***}
N_TT											0.88^{***}

[a]For abbreviations of traits, see Table 2.
***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, non-significant.

(Yoneyama *et al.*, 2007). As strigolactones inhibit tillering, it has been speculated that they could provide a signal to communicate the below-ground nutrient status to the growing shoot (McSteen, 2009). However, nutrient deficiency also directly affects the carbon S/D balance through a negative effect on photosynthesis (Muchow & Sinclair, 1994). Such coordination of the effects of the carbon S/D balance and hormonal pathways on tillering supports the hypothesis that the two pathways are likely to be at least partly under common genetic control.

Model for control of tillering

The observation that the S/D index accounted for most of the environmental effects on tillering allowed an interpretation of the genetic control of tillering. Our study identified two mechanisms for the control of tillering in sorghum: assimilate availability, which explained a major part of the environmental control and some of the genetic control of tillering, and PTT, which explained a major part of the genetic variation in tillering. Based on our findings and those reported previously (Lafarge *et al.*, 2002; Kim *et al.*, 2010a,b; van Oosterom *et al.*, 2011), we propose a framework for the physiological control of tillering that captures both genetic and environmental effects on tillering (Fig. 5).

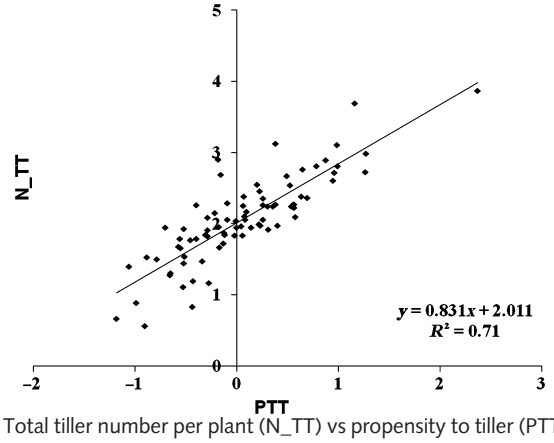

Fig. 4 Total tiller number per plant (N_TT) vs propensity to tiller (PTT) across 85 diverse sorghum (*Sorghum bicolor*) genotypes. For the calculation of PTT, see the Materials and Methods section.

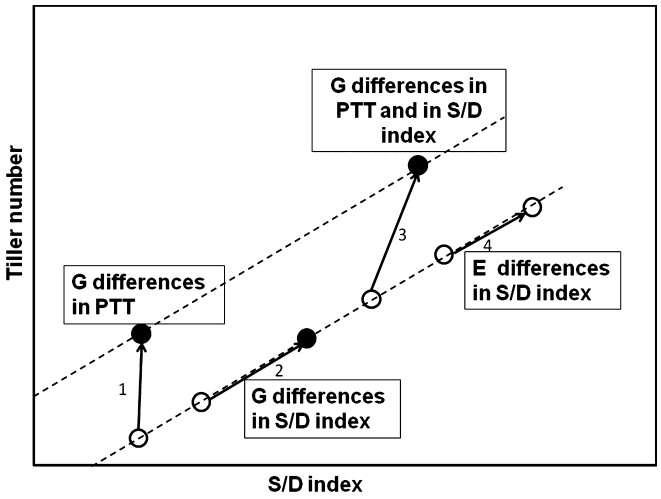

Fig. 6 Schematic representation of the effects of genotypic differences in propensity to tiller (PTT) and supply/demand index (S/D index) and environmental differences in S/D index on the genotype by environment (G × E) interaction for tiller number in sorghum (*Sorghum bicolor*). Arrows 1–3 each connect two different genotypes, grown in a single environment. Genotypes differ in PTT (Arrow 1), S/D index (Arrow 2) or both PTT and S/D index (Arrow 3). Arrow 4 connects a single genotype, grown in two experiments, that resulted in a different S/D index. Open symbols, genotypes with low PTT; closed symbols, genotypes with high PTT.

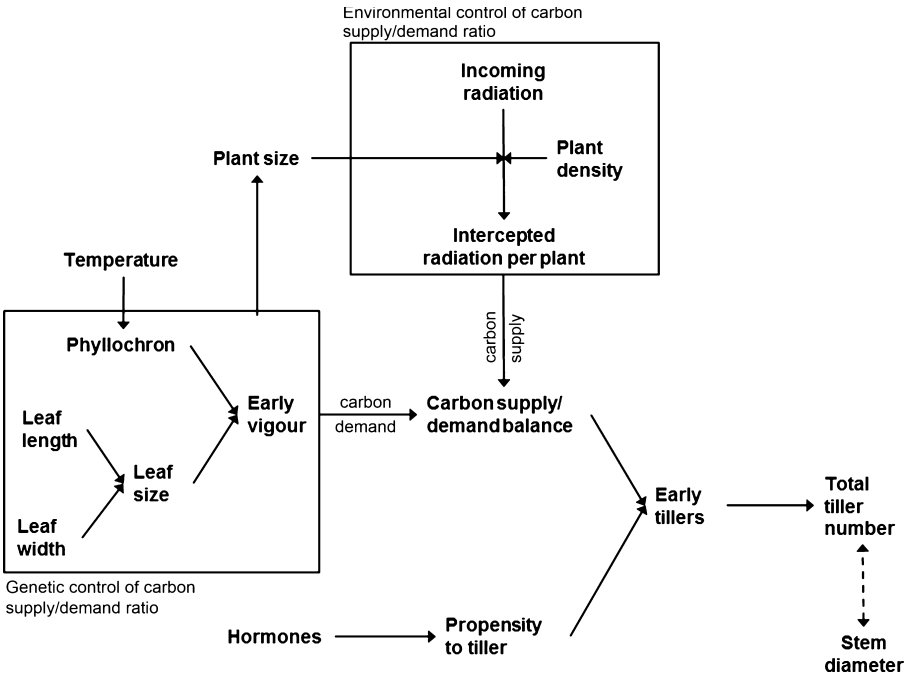

Fig. 5 Physiological framework for the genotypic and environmental regulation of tillering in sorghum (*Sorghum bicolor*). Double-headed dotted arrow between fertile tiller number and stem diameter represents a non-causal relationship.

The carbon demand of the main shoots depends on their early vigour. This is determined by the size and appearance rate of leaves, and the rate of development, which depends on temperature. Carbon supply per plant is a function of plant density and radiation intercepted by the crop, which, in turn, depends on the incoming radiation, leaf area, light extinction coefficient (Lafarge & Hammer, 2002a) and plant density. As carbon availability is most likely to limit tillering during early growth, genotypic and environmental effects on tillering are generally associated with differences in the onset of tillering, and thus with the frequency of appearance of early tillers (Kim *et al.*, 2010a,b). Hence, the carbon S/D framework should capture differences in tiller appearance among pearl millet, sorghum and maize as a consequence of differences in main shoot vigour, which is associated with differences in leaf size. A limitation of the S/D framework, however, is that it only captures the effects of main shoot vigour on the appearance of its subtending primary basal tillers and not of secondary tillers. However, this limitation poses few restrictions in field crops of sorghum, for which the occurrence of secondary tillers is rare. The S/D framework does not capture the cessation of tiller appearance, as this is associated with light quality and, in particular, the ratio of red to far-red light (Casal *et al.*, 1986). The cessation of tiller appearance occurs in sorghum when the leaf area index is *c.* 0.65 (Lafarge & Hammer, 2002b). The rate of tiller mortality in sorghum is highly correlated with the ratio of realized to potential leaf area growth, which reflects the carbon S/D balance of the plant (Lafarge & Hammer, 2002b). The current S/D framework for tiller appearance could thus be extended to capture tiller mortality. Combined with the PTT, this should provide a comprehensive framework to capture effects of environment, species and genotype on the dynamics of tillering.

The effects of the framework of Fig. 5 on the tillering of diverse genotypes across a range of environments are visualized in Fig. 6, where tiller number and S/D index are linearly related for genotypes with a similar PTT, whereas genotypes with contrasting PTT have a similar slope for this relationship, but vary in the threshold S/D index below which tillering ceases (Kim *et al.*, 2010a). In Fig. 6, Arrows 1–3 each connect two genotypes grown in a single environment. Genotypic variation explained by PTT shifts the relationship between tillering and S/D index vertically (Arrow 1). This would represent the situation with SC62C, which had an extremely high PTT compared with all other genotypes included in this study. Genotypic variation in S/D index is captured by moving along a single regression line (Arrow 2), whereas Arrow 3 connects two genotypes that differ in both PTT and S/D index. Arrow 4 connects a single genotype grown across two environments that differ in S/D balance, in a manner similar to Arrow 2. This model captures G × E interactions of a non-crossover type that arise from genotypic differences in the threshold S/D index below which tillering ceases, such as the interaction between R931945-2-2 and Ai4 (Fig. 1), which differed significantly in tillering in Expts 2 and 3, but had each virtually no tillers in Expt 1. The model does not capture the crossover type of G × E interactions, but their importance is relatively minor. This framework provides the basis for the development of a gene-to-phenotype model for tillering that can connect the genetic control of tillering through quantitative trait loci and candidate genes to their phenotypic consequences at the plant or canopy level.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Genotypic values for each trait of sorghum inbred lines, estimated from the three experiments using best linear unbiased predictors (BLUPs)

Table S2 Genotypic values for each trait of sorghum hybrids, estimated from the three experiments using best linear unbiased predictors (BLUPs)

Table S3 Genetic correlations for sorghum traits between experiments and broad-sense heritabilities

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