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**Modelling tiller growth and mortality as a sink-driven process using  
*Ecomeristem*: implications for biomass sorghum ideotyping**

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- **Background and Aims** Plant modelling can efficiently support ideotype conception, particularly in multi-criteria selection contexts. This is the case for biomass sorghum, implying the need to consider traits related to biomass production and quality. This study evaluated three modelling approaches for their ability to predict tiller growth, mortality and their impact, together with other morphological and physiological traits, on biomass sorghum ideotype prediction.
- **Methods** Three *Ecomeristem* model versions were compared to evaluate whether tillering cessation and mortality were source (access to light) or sink (age-based hierarchical access to C supply) driven. They were tested using a field data set considering two biomass sorghum genotypes at two planting densities. An additional data set comparing eight genotypes was used to validate the best approach for its ability to predict the genotypic and environmental control of biomass production. A sensitivity analysis was performed to explore the impact of key genotypic parameters and define optimal parameter combinations depending on planting density and targeted production (sugar and fibre).
- **Key Results** The sink-driven control of tillering cessation and mortality was the most accurate, and represented the phenotypic variability of studied sorghum genotypes in terms of biomass production and partitioning between structural and non-structural carbohydrates. Model sensitivity analysis revealed that light conversion efficiency and stem diameter are key traits to target for improving sorghum biomass within existing genetic diversity. Tillering contribution to biomass production appeared highly genotype and environment dependent, making it a challenging trait for designing ideotypes.
- **Conclusions** By modelling tiller growth and mortality as sink-driven processes, *Ecomeristem* could predict and explore the genotypic and environmental variability of biomass sorghum production. Its application to larger sorghum genetic diversity considering water deficit regulations and its coupling to a genetic model will make it a powerful tool to assist ideotyping for current and future climatic scenario.

**Key words:** *Sorghum bicolor* (L.) Moench, stem biomass, (non-)structural carbohydrates, C source–sink relationships, ecophysiological modelling, ideotyping, tillering, tiller mortality.

## INTRODUCTION

Sorghum is increasingly used as a biomass crop to meet world-wide societal expectations in terms of bioenergy (Regassa and Wortmann, 2014; de Oliveira *et al.*, 2018). It is a C<sub>4</sub> cereal characterized by a high (stem) biomass yield potential and a wide phenotypic diversity for stem biochemical composition (fibre and sugar contents), potentially ensuring the development of diverse value chains (Trouche *et al.*, 2014). Its genetic diversity is also a remarkable source of tolerance to various abiotic stresses, such as drought, which makes it advantageous compared with other crops (Schittenhelm and Schroetter, 2014).

Biomass sorghum is commonly characterized by a long cycle facilitating the production of tall and thick stems. Until now, breeders have not valued the tillering capacity of biomass sorghum, although the maintenance of one or two vigorous tillers until harvest can make a considerable difference in terms of

biomass or grain production (Alam *et al.*, 2014). The end-use value of sorghum biomass depends on its biochemical composition and particularly the partitioning between cell wall components (lignin and structural carbohydrates) and non-structural carbohydrates (Trouche *et al.*, 2014) that varies with the genotype, the environment and the interactions between them (G × E) [sorghum (Perrier *et al.*, 2017; McKinley *et al.*, 2018); miscanthus (van der Weijde *et al.*, 2017); corn (El Hage *et al.*, 2018)]. Several studies showed that stem biomass production and composition and their G × E are not entirely independent, complicating the breeding process (in sorghum: Salas Fernandez *et al.*, 2009; Carvalho and Rooney, 2017; Luquet *et al.*, 2018).

Thus, the design of biomass sorghum ideotypes is challenging and implies multiple morphological and physiological traits, their linkages and plasticity in response to the

environment (Regassa and Wortmann, 2014; Anami *et al.*, 2015; Mathur *et al.*, 2017). In this respect, plant eco-physiological models and functional–structural plant models (FSPMs) can be powerful. They provide a unique approach to describe dynamically the biological processes contributing, at the organ level, to phenotype elaboration depending on  $G \times E$  (Ramirez-Villegas *et al.*, 2015; Chew *et al.*, 2016). They are also increasingly applied to support ideotype design (ideotyping), i.e. the *in silico* exploration of optimal trait combinations in a given environment (Luquet *et al.*, 2016; Yin *et al.*, 2016).

For a given breeding target, model-assisted ideotyping implies that the model used captures realistically the traits where the  $G \times E$  take place and their interactions or trade-offs (Yin *et al.*, 2016). In the case of biomass sorghum and other biomass grass crops, an appropriate model should account for the C source–sink relationships underlying biomass production and quality through C partitioning among sink organs (Dingkuhn *et al.*, 2007) and among structural and non-structural biomass (Perrier *et al.*, 2017). Ideally, light interception and C assimilation should be modelled in order to account, respectively, for plant architectural traits and photosynthetic responses to climate change and fluctuations (Wu *et al.*, 2016). However, considering these formalisms in an ideotyping context still suffers from the difficulty of acquiring calibration data on a large range of genotypes (Parent and Tardieu, 2014; Cabrera-Bosquet *et al.*, 2016) and simpler approaches are still largely used (Wu *et al.*, 2016). Regarding C sink-related traits, to our knowledge, very few models consider C allocation both to the different productive sinks (growing organs) and to their structural vs. non-structural biomass (Dingkuhn *et al.*, 2007). Among these sinks is tillering. Tillering is one of the most plastic yield component traits in grass crops, with high impact on both biomass and grain production (Kumar *et al.*, 2016). It is strongly prone to  $G \times E$ , with respect to not only tiller emergence (Kim *et al.*, 2010a) but also senescence (Buono and Lafarge, 2009). Tillering benefit was much less studied for biomass sorghum, although it is already a key breeding criterion for perennial grass crops [miscanthus (Lewandowski *et al.*, 2016), sugarcane (Gouy *et al.*, 2015)]. Different and not necessarily contradictory approaches were developed to model tillering (response to light quality, C availability or hormonal signals; for a review, see Evers and Vos, 2013). The physiology and modelling of tiller mortality was, however, much less addressed, although its hormonal and nutritional (C and N) control was suggested (Dreccer *et al.*, 2013).

*Ecomeristem* is a plant growth model initially developed to deal with C source–sink relationships underlying the phenotypic diversity and plasticity of rice and sorghum vegetative growth (Luquet *et al.*, 2006). Plant growth is driven by genotypic parameters defining C sink demands and is regulated by a plant internal index of competition for C (supply/demand ratio) and controlling C storage/remobilization. Plant C supply is, however, computed in a simple way at the crop level. *Ecomeristem* concepts were recently extended to the whole crop cycle. This resulted in an underestimated mortality of the youngest tillers, and a bad simulation of culm size and biomass partitioning among culms. Two hypotheses were put forward to tackle this modelling issue: the first one questions the concept of a plant common C pool and suggests that the youngest tillers, at the lowest position within the canopy, are penalized for their

access to light. The second hypothesis is compatible with the concept of a plant C pool but questions the existence of a hierarchical tiller access to plant C supply depending on their age, possibly related to apical dominance processes. To test these hypotheses, two additional *Ecomeristem* versions were implemented and compared.

The present study aims to evaluate several modelling approaches in terms of their ability to capture tiller growth and mortality, and their impact on biomass production and quality, i.e. non-structural vs. structural biomass partitioning, in biomass sorghum. For this purpose, three versions of the *Ecomeristem* model were tested on three field data sets related to two and eight biomass sorghum genotypes. Once validated, the best model version was applied to explore *in silico* optimal trait combinations, i.e. ideotypes depending on targeted productions (fibre and sugar) and cultural constraints (planting density and crop duration). The results are discussed with respect to the challenge of providing modelling tools in support of the pre-breeding process of biomass sorghum.

## MATERIALS AND METHODS

### *Ecomeristem* model

*Ecomeristem* models plant growth at the organ level in the crop stand. It aims to simulate phenotype variability and plasticity within a given species depending on the genotypic (G) and environmental (E: water, light and temperature) regulation of C supply and demand and thus of C source–sink relationships. It represents the crop stand as a population of average plants described by their topology and morphology (organ size and dry weight) with a given homogenous planting density. The topology and morphology are dynamically simulated as the result of  $G \times E$  interactions.

This model was initially created for rice vegetative vigour, and its basic concepts were largely described in Luquet *et al.* (2006, 2007, 2012). A reminder of key concepts is provided below to facilitate the understanding of the novel formalisms implemented for modelling stem elongation and reproductive phases of rice and sorghum. Because of the scope of the present study, modules related to the water regulation of growth will not be addressed. The new formalisms presented below can be used for modelling rice and sorghum by changing parameter values; however, here we explicitly use parameter values related to (biomass) sorghum.

### Basic concepts

**C sink activity.** Thermal time is computed daily and comes as the pacemaker of sink activity (Luquet *et al.*, 2006). ‘Plasto’ (plastochron, °Cd) is a genotypic parameter scheduling the phytomer initiation rate. A phytomer is considered as the ensemble of a leaf, a node and internode, and a tiller bud. At the onset of a given simulation (corresponding to germination), the parameter ‘nbinitleaves’ defines the number of phytomers already created (by analogy with the embryo). Within a phytomer, the leaf is positioned above the internode and related to the latter by the sheath. This parameter is fixed at four for

sorghum. A simulation starts at first leaf emergence (the others are already growing but hidden in the sheath of the first leaf). 'Phyllo' (phyllochron, °Cd) schedules the leaf appearance rate and 'Ligulo' (°Cd), the leaf ligulation rate. 'Ligulo', 'Phyllo' and 'Plasto' together define the duration of different phases of organ growth, particularly the leaves (see Supplementary Data Fig. S1A for the developmental framework of sorghum phytomers and Fig. S1B for leaf growth phases: phase 1, from leaf initiation to its outgrowth from the sheath of the previous leaf; phase 2, from leaf tip appearance to ligulation). In sorghum, 'Ligulo' is longer than 'Phyllo', making the number of visible expanding leaves 2–4. At each new 'Plasto', a tiller bud is activated on culms carrying at least four visible leaves. During the vegetative phase, only the leaves are growing. The first leaf length is defined by an initial genotypic parameter, 'Lef1' (cm). The specific parameter 'wlr' translates leaf length into width, and the specific parameter 'LL\_BL' defines the ratio between blade and sheath length. An allometric coefficient ('Allo\_Area') is used to compute blade area from width and length. 'FSLA' (first leaf-specific leaf area, cm<sup>2</sup> g<sup>-1</sup> blade) is fixed for a given genotype or species (initial parameter based on measurements) and used to translate the first leaf area into dry weight.

Leaves on a given culm are then initiated successively each time the cumulated thermal time completes a new 'Plasto'. The final length of the newly activated leaf is computed (pre-dimensioned) at this time, based on the previous leaf length plus an additive parameter MGR (meristem growth rate, cm). Leaves on tillers are dimensioned similarly but considering the average of the previous leaf on the main stem and on itself (see Luquet *et al.*, 2006 for details). Based on the translation of daily leaf growth into dry weight using structural SLA (specific leaf area evolving with leaf rank as detailed in Luquet *et al.*, 2006), a daily C demand for growth is computed at the whole-plant level ('DayDemand'). 'DayDemand' includes root demand until panicle initiation, computed proportionally to the shoot demand in a decreasing way (see Luquet *et al.*, 2016 for details).

*C source activity and source-sink regulation of growth and development.* Each day, leaf blade area is summed on the plant (PLA) and aggregated at canopy level (LAI using a parameter defining plant density m<sup>-2</sup>). On this basis, Beer-Lambert and Monteith equations are run at canopy level to compute, respectively, light interception and C assimilation rates and finally plant daily C supply availability, as described in Luquet *et al.* (2006).

The ratio between the daily plant C supply and demand is computed and named IC, a plant state-variable index of plant internal competition for C resource. On a given day, IC is averaged over the previous period corresponding to the duration of one 'Plasto' in order to smooth its kinetics and related morphogenetic responses.

(1) If supply exceeds demand (IC > 1), the C excess is stored in vegetative tissues to a maximum amount of 50 % of leaf structural mass (70 % for the internode which will be described later); this makes SLA an emerging property of the model, computed from structural (see structural SLA computation above) and non-structural leaf biomass; if the excess cannot be entirely stored, the surplus is spilled-over and considered as lost as this surplus did not show any benefit to root growth in a previous study (Gutjahr *et al.*, 2013).

(2) If IC is < 1, the pool of C stored is mobilized and, if it is not enough to afford the daily C demand, growth is reduced, the senescence of the oldest leaf of each culm is accelerated and 40 % of its structural biomass is remobilized to the C pool.

Growing organs are at this stage not prioritized for their access to plant C supply that is partitioned uniformly to each of them proportionally to their demand. In addition, IC comes as a regulator of three plant morphogenetic events: (1) if IC is < 1 at the time of initiation of a phytomer, its pre-dimensioning is reduced proportionally (by multiplying potential pre-dimensioning by  $\sqrt{IC}$ ); (2) if IC is < 1 during organ growth, daily sink activity (elongation rate and resulting daily demand) is reduced proportionally (by multiplying the potential elongation rate by  $\sqrt{IC}$ ); (3) if IC is < IC<sub>t</sub> (genotypic threshold parameter tested on IC averaged during phase 1 of expansion of a given leaf), then the young tiller outgrowing from the corresponding bud is killed. Otherwise, the tiller keeps on growing. This test is performed only once during the life of the tiller.

#### Model concept extrapolation to the whole plant cycle

*Scheduling of main developmental phases.* To make the onset of stem elongation, panicle initiation and changes in leaf development rates and sizing independent (see Supplementary Data Fig. 1A), three ontogenetic stage parameters were implemented:

(1) 'NbleafParam2' indicates the number of leaves which have appeared on the main stem when some morphogenetic rules change: 'Plasto', 'Phyllo' and 'Ligulo' are increased; the size increment of successive leaves (MGR) is modified as is the blade to sheath length ratio ('LL\_BL'). For this purpose, the following genotypic parameters were created:

$$X = X \times C_x \quad (1)$$

$$R_{LB} = R_{LB} + S_{LB} \times n \quad (2)$$

where  $X$  is either MGR, 'Plasto', 'Phyllo' or 'Ligulo',  $n$  is the rank above 'NbleafParam2' of the considered organ,  $S_{LB}$  the slope for the 'LL\_BL' function ( $R_{LB}$ ) and  $C_x$  is the specific coefficient for 'Plasto', 'Phyllo', 'Ligulo' or MGR. This is applied on all tillers at the same time.

(2) 'NbLeaf\_StemElong' indicates the number of ligulated leaves on the main stem at which internode elongation starts. At this stage, the internode belonging to the phytomer bringing the youngest ligulated leaf on the main stem starts elongating. Simultaneously internode elongation starts on other tillers that bring at least one ligulated leaf. The internode length and diameter are pre-dimensioned as follows:

$$L_I(n) = L_L(n) \times R_{LI} \times \sqrt{IC} \quad (3)$$

$$L_I(n) = L_I(n-1) \times S_I \times \sqrt{IC} \quad (4)$$

$$D_I(n) = D_I(n-1) \times C_D \times \sqrt{IC} \quad (5)$$

with  $L_I(n)$  and  $D_I(n)$  the pre-dimensioning, at phytomer  $n$  of internode length and diameter, respectively,  $L_L(n)$  the

pre-dimensioning of leaf length, and  $R_L$ ,  $S_i$  and  $C_D$  genotypic parameters. If internode rank is below 'Nbleafparam2', eqn (3) is used and otherwise eqn (4) for internode length pre-dimensioning.  $\sqrt{IC}$  has an upper limit equal to 1 in all equations.

The internode expansion rate is computed similarly to leaves assuming a similar sensitivity to IC and a duration of 3 'Ligulo' in order to have three internodes expanding at the same time, as suggested by Nakamura *et al.* (2011). Daily internode length and width enable computation of the internode volume, assuming internodes are cylindrical. Based on two parameters defining internode volume density (g of structural biomass cm<sup>-3</sup>), the daily internode growth is translated into a daily C demand. For a given internode, its density evolves linearly along its elongation from 'density\_IN1 to density\_IN2' (up to 0.25 for sorghum).

- (3) 'MaxLeaves' indicates the rank of the flag leaf phytomer on the main stem, i.e. the last vegetative phytomer initiated, before the phytomer bringing the panicle and the peduncle is initiated. Accordingly, panicle initiation occurs on the main stem when the number of 'Plasto' reaches 'Maxleaves' + 1. The panicle dimensioning (spikelet number per panicle) is then onset until flowering as follows:

$$S_p(t) = S_p(t-1) + R_s \times D(t) \times \sqrt{IC} \quad (6)$$

where  $S_p(t)$  is the number of spikelets per panicle on a given day  $t$ ,  $R_s$  is the spike creation rate and  $D(t)$  is the total degree-days of that given day  $t$ .

Onset of panicle initiation occurs first on the main stem and one 'Plasto' later on all tillers made of at least one phytomer with a fully expanded leaf. The other tillers will remain sterile. As tillers grow out later than the main stem and because flowering (FLO) is synchronized among all culms on the plants, fertile tillers systematically bring fewer leaves than the main stem. Based on another genotypic parameter defining spike number per panicle length unit, a panicle length is computed (not shown).

When the last leaf (flag leaf) is expanded on the main stem, the last internode and the peduncle are activated and start growing simultaneously, which starts the pre-flowering stage. The peduncle elongates during this phase, lasting one 'Ligulo' for sorghum. The peduncle is pre-dimensioned as a function of the last internode length and a multiplicative coefficient (equal to 1.5 in sorghum), regulated by IC (similarly to other organs). Accordingly, panicle exertion is completed at the end of the pre-flowering period that is considered to correspond to the flowering stage (FLO).

At FLO, the grains start filling to a rate defined by a genotypic parameter ('Grain\_filling\_Rate', in g °Cd<sup>-1</sup>) that can be regulated by  $\sqrt{IC}$  as well. A number of 'Plasto' (six for sorghum) defines the grain-filling phase duration. Grain physiological maturity occurs one 'Plasto' after the end of the grain-filling phase.

During the reproductive phase (from panicle initiation time), tillering is still tested but only in the case where C supply remains positive after the daily demands for growing organs are afforded, in order to represent an accentuated apical dominance. This, associated with the fact that IC decreases dramatically

during stem elongation and grain-filling phases due to an increase of C demands, makes tillering inhibition an emerging property of the model.

#### Leaf senescence

In the first version of *Ecomeristem* (Luquet *et al.*, 2006, 2012), leaf senescence was activated only when plant C storage reached zero. This underestimated leaf and tiller senescence dynamics when using the model for the whole-plant cycle. A leaf senescence module was thus implemented considering a linear senescence rate from leaf ligulation until it reaches a maximal age defined by the genotypic parameter 'Coeff\_life\_span' (in °Cd) at which it is entirely senesced (see Phase 3 in Supplementary Data Fig. 1B). Each day, the portion of senesced leaf is partially allocated to the senesced dry weight compartment and remobilized to the C storage pool, as described above.

#### Individualization of tiller growth and senescence

From the onset of stem elongation, up to grain physiological maturity, C balance, growth and senescence computation is individualized per culm (main stem and tillers) in order to capture the desynchronization of tiller development (panicle initiation and internode elongation), related C source-sink relationships and (leaf and tiller) senescence processes. Three approaches were compared.

*Approach 1: the daily plant C pool is partitioned equitably among culms proportionally to their green leaf fraction.* For the vegetative phase, *Ecomeristem* (Luquet *et al.*, 2006) considers that all growing organs have an equitable access to a common C pool (supply and storage) computed at the plant level, without any prioritization as to whether they belong to the main stem or a tiller. This approach was first extrapolated to the subsequent developmental phases. For this purpose, the daily C supply of a given culm is computed as the fraction of the plant C supply multiplied by the ratio between tiller and plant green leaf biomass:

$$S_{ij} = S_i \times (B_{ij}/B_i) \quad (7)$$

where  $S$  is the supply of the  $j$ th tiller for the  $i$ th day,  $S$  the plant supply for the  $i$ th day,  $B$  the total leaf biomass of the  $j$ th tiller for the  $i$ th day, and  $B$  the total plant leaf biomass for the  $i$ th day.

The daily C demand on a given culm is computed similarly to that described above. A local IC and C storage is then computed at the level of each culm to locally control C remobilization, growth reduction and leaf senescence, similarly to that explained at the whole-plant level.

The approach was, however, not able to simulate biomass sorghum growth and particularly biomass partitioning after stem elongation onset. Indeed, although plant total biomass was correctly simulated, the partitioning between main stem and tiller growth (and tiller abortion) was not well represented, main stem growth and height being underestimated to the benefit of tillers (see Fig. 1). An underestimation of C supply allocated to the main stem should be explained either by a bad

partitioning of light interception among culms due to the use of the Beer–Lambert law (see Approach 2) or by a bad estimation of C sink strength of each culm (see Approach 3). To test these two hypotheses, two further approaches were implemented and compared.

**Approach 2: a light interception model enabling a better spatial distribution of light and C supply among culms.** In this approach, the Beer–Lambert law that computes light interception at the canopy level and the concept of a common C pool for the whole plant are questioned. Indeed, the main stem (and the oldest tillers), being older and taller, may be at an advantage for their access to light and C assimilation and thus grow better. In this respect, a model computing light interception per culm was tested, inspired from that proposed by Yin and van Laar (2005). Before canopy closure (tested according to inter-row space and the length of the largest leaf on the plant; the canopy is considered closed when the length of the largest leaf is greater than half of the inter-row space), light interception is computed per horizontal canopy layer considering whether leaves belonging to this layer are sunlit and shaded leaves and taking into account the inter-row spacing. If the canopy is closed, light interception is computed considering sunlit or shaded fractions of leaf area per continuous horizontal canopy layer. For both periods (open or closed canopy), light interception is discretized per leaf to compute the C assimilation for each culm.

**Approach 3: a hierarchical culm access to the whole-plant C pool depending on their age.** This approach assumes that the

oldest culms are generally more prone to be fertile. For this purpose, the implemented approach considers culms hierarchically (from the oldest to the youngest ones) in order to supply C locally, from the plant C pool, proportionally to the local daily C demand, when possible. IC and the resulting regulation of organ growth, senescence, C storage/remobilization and ultimately tiller death are thereafter computed similarly to Approach 1. Accordingly, the younger a tiller, the smaller the amount of C supply potentially provided. The youngest tillers are thus more prone to be source limited and to senesce. It must be mentioned that the daily C storage in leaf tissues is still considered but intervenes only in the case where the C storage in the internodes is maximal. Figure 1 summarizes the way Approach 3 models tiller C source–sink relationships.

*Experimental data*

Three experiments were carried out in the DIAPHEN field phenotyping platform at Mauguio (South of France; Delalande et al., 2015) during the summer seasons 2014, 2015 and 2016 (sowing on 23 May, 13 May and 18 May, respectively). Two to eight biomass sorghum genotypes were studied. Germination was considered as the appearance of the tip of the first leaf out of the soil; it occurred on average 5 d after sowing in the three experiments. Each experiment consisted of a randomized complete block design with three replications.

The 2016 experiment consisted of comparing two biomass sorghum genotypes of similar height but contrasting

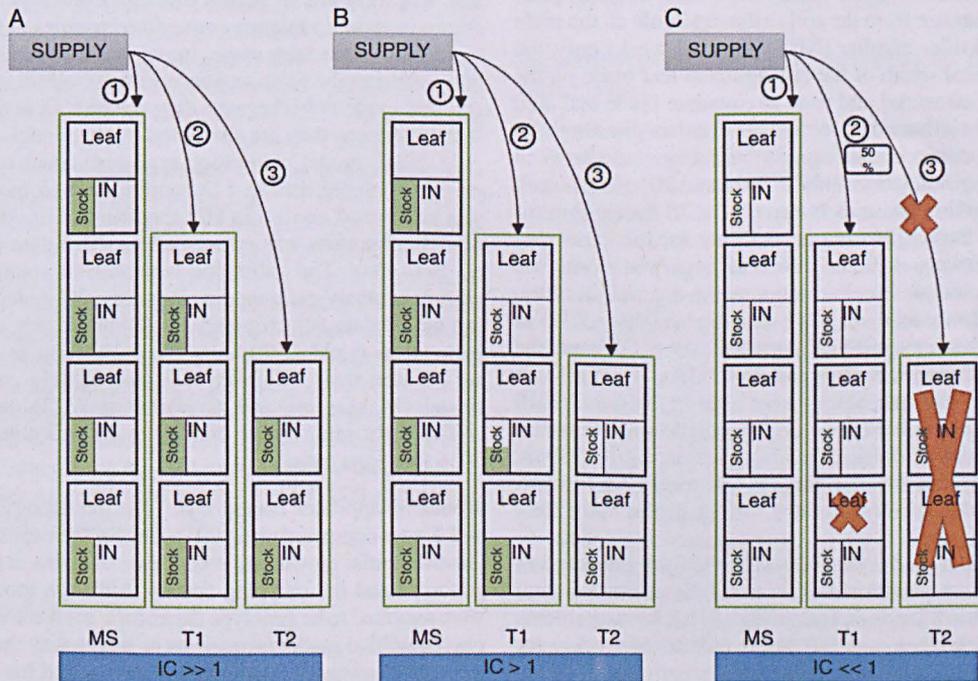


Fig. 1. Schematic representation of the apical dominance concept in *Ecomeristem* (modelling Approach 3). Demands are satisfied subsequently according to tiller age from the oldest to the youngest, beginning with the main stem. If C supply is higher than C demand (case A), all demands are satisfied and the remaining C supply is stored in internodes, then leaves. If C supply is close to demands (case B) all demands are satisfied but the internode-available C storage compartment is not entirely filled (leaf C storage is not filled at all) and can even be remobilized. If C supply is much less than the C demand (case C), demands are satisfied according to tiller age; tillers that meet a C deficit (C supply lower than the demand) will have their oldest leaf killed, and tillers with only one leaf remaining will die.

morphology and tillering patterns (BIOMASS140, a commercial hybrid; and IS 26833, a *caudatum* line, named G1 and G6, respectively, in this study; see details in Luquet *et al.*, 2018) at two cropping densities: a classical planting density with 0.8 m row spacing resulting on average in a plant density of 20 plants  $m^{-2}$ . This cropping density will hereafter be named LD (low density); a high cropping density (HD), with inter-row spacing reduced to 0.4 m, resulted in a plant density of 40  $m^{-2}$ . This experiment aimed to compare the three modelling approaches described above with respect to their capacity to capture biomass sorghum phenotypic plasticity particularly in terms of tiller growth and mortality dynamics. In this experiment, one block consisted of three rows (LD) or six rows (HD) of 4 m.

The 2014 and 2015 experiments were used to validate *Ecomeristem* in its capacity to simulate the growth, development and biomass component traits of eight biomass sorghum genotypes (hereafter named G1–G8 including G1 and G6 studied in 2016) representative of the morphological diversity found in the sorghum diversity reference set (organ size, number, tillering, stem fibre vs. sugar content; see details in Luquet *et al.* (2018)). In these experiments, plants were cropped only at LD, and one replication of one genotype consisted of four, 8 m long rows. Details on field monitoring and measurements are provided in Luquet *et al.* (2018) and only key information is provided here. In the three experiments (2014, 2015 and 2016), four plants per block were tagged by marking the fifth leaf with a black point when plants presented an average of seven visible leaves. These plants were thereafter monitored weekly to measure the number of appeared (APP), ligulated (LIG) and green leaves on the main stem (a leaf is considered green when <50 % senesced), the plant height, i.e. the distance from the soil to the top ligule on the main stem (PHT), and tiller number (NBT). Every 2 weeks only, the length and maximal width of the last ligulated leaf blade on the main stem were measured and used to calculate blade leaf area (AREA) using an allometric coefficient constant for sorghum (0.69). On three dates: beginning of stem elongation (between 40 and 45 d after germination, only in 2015 and 2016); last week of the stem elongation phase (between 60 and 70 d after germination); and final harvest (during grain filling for the genotypes that flowered, between 110 and 130 d after germination), 3–4 plants were sampled per block to estimate the dry weight of the different organs [only total above-ground dry weight (ADW) in 2015 at the first date]: stem (SDW), green leaves (LDW) and the panicle (PDW) of the main stem and bulk tillers (TDW). Main stem sub-samples (one per block) were used in 2014 and 2015 for NIRS (near infrared spectroscopy) prediction of stem biochemical composition [detailed in Luquet *et al.* (2018)]. Only the prediction of soluble sugar content was used here to compute non-structural carbohydrate dry weight in the main stem (MSS, g).

In 2016 only, and on the same three dates, light interception measurements were performed using a SunScan canopy analysis system (Delta-T Devices, Cambridge, UK). Measurements were taken in each block near the plants monitored weekly for development and phenology, keeping the sensor parallel to the soil and at three levels: within the canopy (above, at medium height and at ground level), by positioning the SunScan along the row at the base of the plants, and with a 45° angle on the left and right sides of the row. At final harvest, the number of internodes, their cumulated length, the length of the peduncle and

the diameter of the first and the last expanded internodes were measured on the main stem.

Air temperature ( $T_a$ ), relative humidity and photosynthetically active radiation (PAR,  $MJ\ m^{-2}$ ) were measured hourly using a Cimel516 meteorological station (CIMEL electronic, Paris, France). Estimated daily  $T_a$  and PAR were used as input values for simulations with *Ecomeristem*. The water balance module was not activated in the model, considering that no water deficit event occurred.

#### Modelling and simulation experiments

**Modelling calibration.** A genetic algorithm (Luquet *et al.*, 2016) was used to carry out model parameter estimations. It consists of a metaheuristic method searching for the set of parameters maximizing the accuracy of simulations for several available phenotypic traits: APP, LIG, AREA, ADW, MSDW, MSS, PDW and PHT. The accuracy of parameter estimation was computed as the root mean square error [RMSE, expressed as a percentage in eqn (8)] between simulated and observed data. The set of parameters that was estimated, measured or fixed (species dependent) is summarized in Table 1.

$$NRMSE (\%) = \sqrt{\frac{\sum_{i=1}^n \left( \frac{y_i - \hat{y}_i}{y_i} \right)^2}{n}} \quad (8)$$

It must be mentioned that this RMSE used a weighing coefficient to give increasing importance to error values with plant age: this weight is increased by 10 % every day. This was necessary in order to tolerate some discrepancies at the early stage more than at the later stage, in particular regarding tiller number, as during the tillering phase some small tillers could be not counted in the field (because they were about to die or were too small) whereas they are simulated by the model.

In 2016, model calibration was performed for G1 and G6 on experimental data in LD conditions, and model validation was performed on data in HD conditions. For other genotypes, model calibration was performed on 2015 data and validation on 2014 data. The validation consisted of running the model with previously estimated parameters and independent meteorological data (or cropping conditions). Only two genotypic parameters could differ from the calibration to the validation set because their variability was not entirely captured by the model: 'MaxLeaves' and 'CoeffInDiamW' (Table 1).

The light interception model could be calibrated only on 2016 SunScan data.

**Modelling approach comparison.** The modelling approaches 1, 2 and 3 were compared using 2016 data for their capacity to capture the differential growth and senescence of culms depending on the genotype and the cropping density. Although model parameters were assumed to be genotype dependent, the three approaches implied modified model formalisms in such a way that a calibration (i.e. model parameters estimation) was needed for each approach in order to compare them. The model comparison therefore concerned the behaviour of each approach at the optimum, i.e. for a set of parameters that gives the best results with respect to observation points, assuming the model parameters are not only genotypic dependent but also model dependent.

TABLE 1. Estimated, fixed (\*) and measured (\*\*) parameters with, when required, the letter code used for illustrations in this article and the range used for parameter estimation (†) or obtained by parameter estimation and used for eight of them for sensitivity analysis (‡)

Parameter	Letter code	Description (unit)	Interval/value
Epsib	A	Light conversion coefficient (g MJ <sup>-1</sup> )	[3.0, 8.0]† [5.0, 8.0]‡
Ict	B	Threshold parameter tested on IC to enable a culm to live	[0.5, 2.5]† [1.0, 2.5]‡
MGR_init	C	Initial value of the additive parameter meristem growth rate for leaf length pre-dimensioning	[6.0, 14.0]† [6.7, 10.3]‡
slope_LL_BL	D	Slope for the leaf length to blade length ratio decrease after Nbleafparam2	[0.0, 0.4]† [0.0, 0.3]‡
slope_length_IN	E	Multiplicative coefficient to compute internode n final length from n - 1 after Nbleafparam2	[0.85, 1.0]† [0.85, 1.0]‡
CoefflinINDiam**	F	Basal main stem diameter (cm)	[1.3, 2.6]‡
density_IN2	G	Internode final volume density (g cm <sup>-3</sup> )	[0.08, 0.3]† [0.08, 0.17]‡
Plasto/phylloligulo		Initial values of plastochron, phyllochron and ligulochron (°Cd)	[25.0, 45.0]† 25/30/35‡
Nbleafparam2*		Number of leaves which appeared on the main stem when some morphogenetic rules change	15
NbLeaf_StemElong*		Number of ligulated leaves on the main stem when internodes start elongating	8
Maxleaves*		Final number of phytomers on the main stem	[17, 27]

*Sensitivity analysis and ideotype exploration.* A sensitivity analysis was carried out using seven key genotypic parameters, i.e. those with the highest genotypic variability in the present study (Table 1) using an extended-FAST method (see Luquet *et al.*, 2012 for details). This method changes the value of only one parameter at each step and evaluates how it affects an output variable. It computes the direct and interactive effects of each considered parameter on a targeted simulated variable. This analysis was performed on two types of biomass output of potential interest for bioenergy production: plant stem structural ('fibre') and non-structural (sugar or C stored) biomass, produced at two planting densities in order to study further the added value that tillering should provide: 10 and 20 plants m<sup>-2</sup>, corresponding to that commonly met in West Africa and Europe, respectively. This was performed for one type of phenology corresponding to that typical of biomass sorghum: a long cycle genotype (130 d after germination to reach the grain-filling stage) producing 23 leaves. The development rate ('Plasto', 'Phyllo' and 'Ligulo') was, however, considered as constant and fixed at the values estimated for G1 (typical biomass hybrid, international commercial reference; a discretization length of 100 000 was used as a sample size, i.e. 100 000 samples for each parameter, resulting in 700 000 simulations for each configuration: planting density × targeted biomass output). In order to provide a first appraisal of ideotypes for each type of production, the ten best parameter sets maximizing a targeted production were selected among the 700 000 simulations and their parameter values were analysed.

#### Programming and software

*Ecomeristem* is programmed in C++ (<https://dl.acm.org/citation.cfm?id=2543987>). All equations implemented in *Ecomeristem* are available at [http://biomasorgho.cirad.fr/ecomeristem2\\_doc/](http://biomasorgho.cirad.fr/ecomeristem2_doc/). All figures were constructed with R software and the ggplot2 R package (<https://cran.r-project.org/web/packages/ggplot2/>) or Excel software. Parameter estimation was performed using the differential evolution algorithm

implemented in the 'DEoptim' R package (<https://cran.r-project.org/web/packages/DEoptim/>). Sensitivity analysis was performed using the extended-FAST method implemented in the 'sensitivity' R package (<https://CRAN.R-project.org/package=sensitivity>).

## RESULTS

#### Comparison of the three modelling approaches for C partitioning among culms

Once the light interception model used in Approach 2 was calibrated on 2016 SunScan data (not shown, error of 9.8 % for Beer-Lambert and 3.5 % for Approach 2), the three modelling approaches were compared on 2016 data. This is presented in Fig. 2. As expected, Approach 1 was unable to capture tiller mortality and biomass partitioning amongst culms as observed in the 2016 experiment in LD and HD conditions. The mean RMSE over four phenotypic traits reached 54.3 %, with the biggest errors made on tiller number. Approach 2 did slightly better for PHT and MSS, due to a better partitioning of light and C supply among culms depending on their spatial distribution, but worse on ADW, with a mean RMSE of 57.2 %. However, both Approaches 1 and 2 underestimated MSDW and overestimated ADW, which can be largely explained by the absence of tiller abortion. Approach 3 gave a significant improvement (mean RMSE of 8 %) with a perfect fit on NBT. Logically only Approach 3 provided consistent validation results using data in HD conditions. This is illustrated for genotype G6 in Fig. 3. To further understand how plant C supply partitioning among culms is simulated differently in each modelling approach, simulations were compared on a given day in identical simulation conditions. This is presented in Table 2. Interestingly, by considering the geometrical position of tillers within the canopy and their resulting reduced access to light, Approach 2 simulated a

smaller proportion of plant C supply for tillers compared with Approach 1. However, this was obviously not enough to simulate tillering cessation and mortality correctly (Fig. 2). Approach 3 simulated a lower proportion of plant C supply to tillers that resulted in a realistic simulation of mortality thereafter. Accordingly, only Approach 3 will be considered hereafter.

#### Modelling the phenotypic variability of biomass sorghum genotypes

Model calibration was carried out on the eight genotypes studied in the 2015 experiment. The results are presented in Fig. 4, showing the ability of *Ecomeristem* to simulate a large range of biomass sorghum phenotypes (mean RMSE of 10.4 % per genotype, per output variable). For all genotypes, *Ecomeristem* was able to simulate tillering dynamics and mortality (if any). Errors on early tiller number were, however, higher, which can be explained by the fact that model simulations account for very small tillers not necessarily measured in the field. Interestingly, *Ecomeristem* was able to simulate the dynamic allocation of C to structural and non-structural (sugars) stem biomass, resulting from the complex C source–sink relationships involved within the plant (Fig. 4D).

Figure 5 presents *Ecomeristem* validation on data from the 2014 experiment. Only 'MaxLeaves' and 'CoefflinINDiam' were modified from the calibration to the validation set of genotypic parameters, although these parameters did not change significantly from one year to the next. The results show the ability of the model to predict fairly well the component traits of stem biomass production and properties. The main weakness concerns the prediction of the highest tiller numbers, which corresponds to the end of the tillering phase, the time at which the model can simulate the creations of small, unviable tillers, not necessarily observed experimentally.

#### Model sensitivity analysis and ideotype exploration

Figure 6 shows the direct and interaction effects of each genotypic parameter on the simulation of structural (upper row) and non-structural (lower row) biomass at two planting densities: 10 and 20 plants m<sup>-2</sup>. The proportion of direct effects to the total effect of parameters A (light conversion coefficient, 'Epsib'), F (basal diameter) and G (internode density) were higher at 10 than at 20 plants m<sup>-2</sup> (only on structural biomass for parameter A). This is the result of smaller inter- and intraplant competition

at this lower planting density making the direct effect stronger for these key C supply and demand parameters. These parameters remained, however, the most influential parameters in all situations. Parameters B (ICt, tillering sensitivity to C availability) and C (MGR, leaf sizing) became more influential at higher planting density. These parameters in the first place control the number and the size of leaves set up during the vegetative phase and therefore the capture of light. Neither the parameter controlling the length increment of successive internodes (E) nor the blade area decrease rate for the top leaves from 'NbleafParam2' upward (D) exhibited a strong effect whatever the planting density and the targeted production. This can be easily explained for parameter E by its low genotypic variability or for parameter D by the minor effect of top leaf size for genotypes that exhibited an average high light conversion efficiency.

The ten sets of parameters simulating the genotypes maximizing the structural or non-structural biomass production, for each planting density, were then analysed. They are presented in Fig. 7. In both cases, several ideotype profiles emerged, although stem biomass yield (per hectare) was stable among ideotypes in a given configuration (plant density × targeted production) and between the two densities or the two targeted productions (Supplementary Data Fig. 2). Light conversion efficiency (parameter A) exhibited consistently high values across ideotypes for the four configurations, whereas other parameters showed variability among configurations and between ideotypes in a given configuration. At 10 plants m<sup>-2</sup> and considering stem structural dry weight as targeted production (Fig. 7A), tillering sensitivity to C availability (parameter B), top leaf blade area downsizing (parameter D) and internode volume density (parameter G) were the most variable, whereas the parameters controlling internode length and diameter (parameters E and F) and leaf size profile (MGR) were fairly high. At 20 plants m<sup>-2</sup> for the same targeted production (Fig. 7B), two profiles emerged. The first profile favoured tillering (low parameter B, i.e. low sensitivity of tillering to C) together with a high internode density (parameter G); the second one observed high parameter B (high sensitivity of tillering to C) and low density. However, whatever the profile, all ideotypes ended up with the same tiller number and contribution to production (Supplementary Data Fig. 2). The same trend could be observed between these two parameters with stem non-structural C dry weight as the targeted production. In addition, in this configuration and for both planting densities, high internode density (parameter G) pairs with a low downsizing of top leaf blade area (parameter D) (Fig. 7C, D).

In general, D20 ideotypes maximizing stem dry weight exhibited small leaves (parameter C) and D20 ideotypes maximizing stem non-structural C dry weight exhibited a low ICt

TABLE 2. Amount of the plant C supply allocated to each culm computed by the three modelling approaches compared in this study

	Mainstem supply (%)	Tiller 1 supply (%)	Tiller 2 supply (%)	Tiller 3 supply (%)
Approach 1	34.19	27.39	23.74	14.68
Approach 2	45.47	24.15	18.75	11.63
Approach 3	57.39	33.48	9.12	0

Example of one genotype (G1), one planting density (20 plants m<sup>-2</sup>) and one year (2014 meteorological data) on one date (3 July 2014) on an identical average plant during the phase of tillering cessation.

Results are expressed as the percentage of total plant carbon supply.

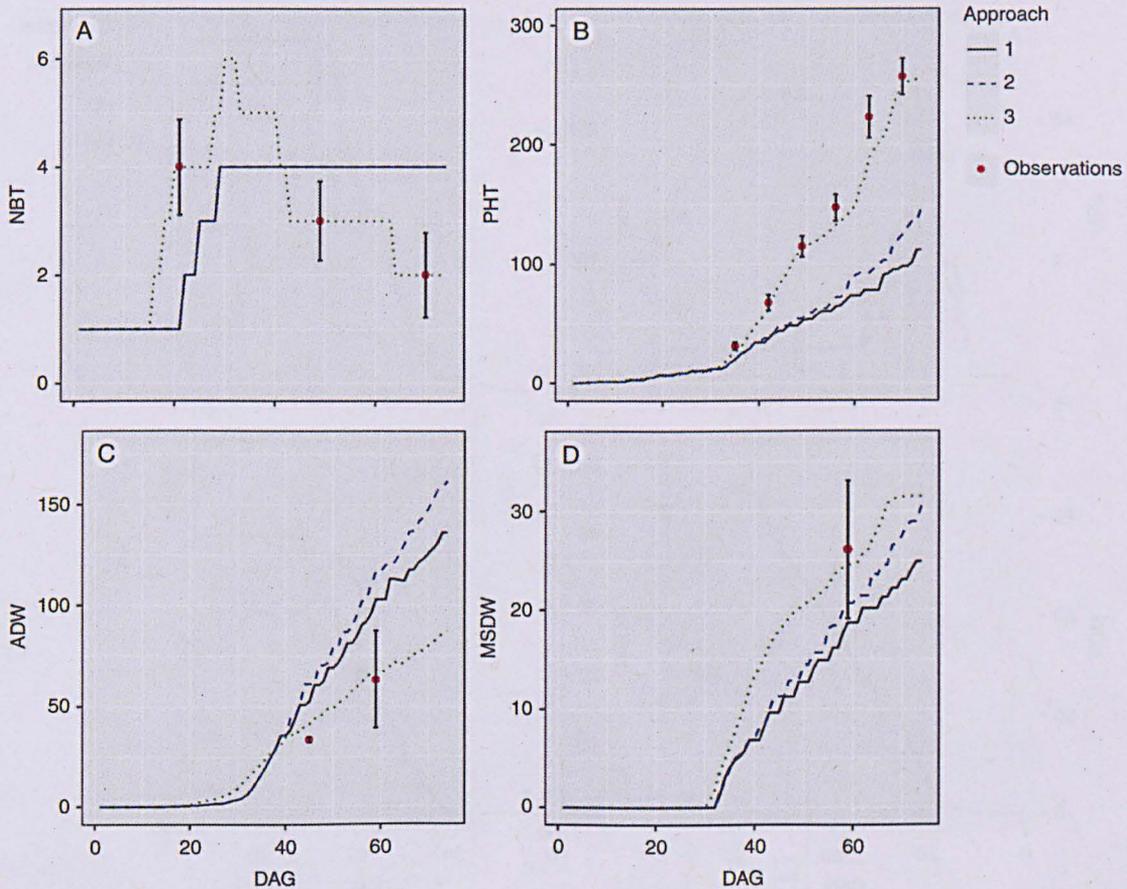


FIG. 2. Comparison of the simulations with *Ecomeristem* Approach 1, Approach 2 and Approach 3 of: (A) NBT (tiller number), (B) PHT (plant height in cm), (C) ADW (above-ground dry weight in g) and (D) MSDW (main stem dry weight in g) for genotype G1 in the 2016 experiment. Red dots indicate field observation means with standard error bars. DAG: days after germination.

value with high internode diameter (parameter F) and length (parameter E). Tillering capability showed more variability considering plant stem dry weight than stem non-structural biomass as the targeted production, but this resulted in a similar contribution of tiller to final production.

For each condition, the mean value over the ten ideotypes of key stem biomass production variables as well as of stem biomass yield per hectare was computed (see Supplementary Data Fig. 2). It is interesting to note that, at lower density, tillering was able to compensate either stem structural or non-structural biomass production, leading to stable production between the two densities used.

## DISCUSSION

The present study aimed to (1) evaluate three modelling approaches for their ability to predict tiller growth and mortality and (2) use the best approach for evaluating the impact of tillering traits, among other morphological and physiological traits, on biomass sorghum production and quality (non-structural vs. structural biomass partitioning) depending on cropping density in an ideotyping context. The sink-driven modelling approach considering a hierarchical access of tillers to C resources depending on their age was shown to be the most efficient. The

results discussed below come as a proof of concept of its interest for a (pre-)breeding context and open the door to its application for analysing larger genetic and environmental diversity.

### *Novel insights for modelling the $G \times E$ underlying biomass sorghum production*

*Advantage of considering plant C source-sink relationships at organ and culm levels.* Three versions of *Ecomeristem* were compared to evaluate whether tiller growth, mortality and final contribution to biomass production was C source (light interception distribution within the plant) or sink (apical dominance) driven. It could be suggested that, in a given planting situation, the genotypic variability in tiller final contribution to stem biomass yield was mainly explained by the level of plant internal competition for C among culms and the existence of a prioritization related to their age. This should be attributed to hierarchical apical dominance of tillers, apical dominance being known to regulate the allocation of resources within the plant (Bennett *et al.*, 2012); however, we did not check in the present study whether a hormonal phenomenon was indeed driving these phenotypes. The processes underlying tillering and particularly

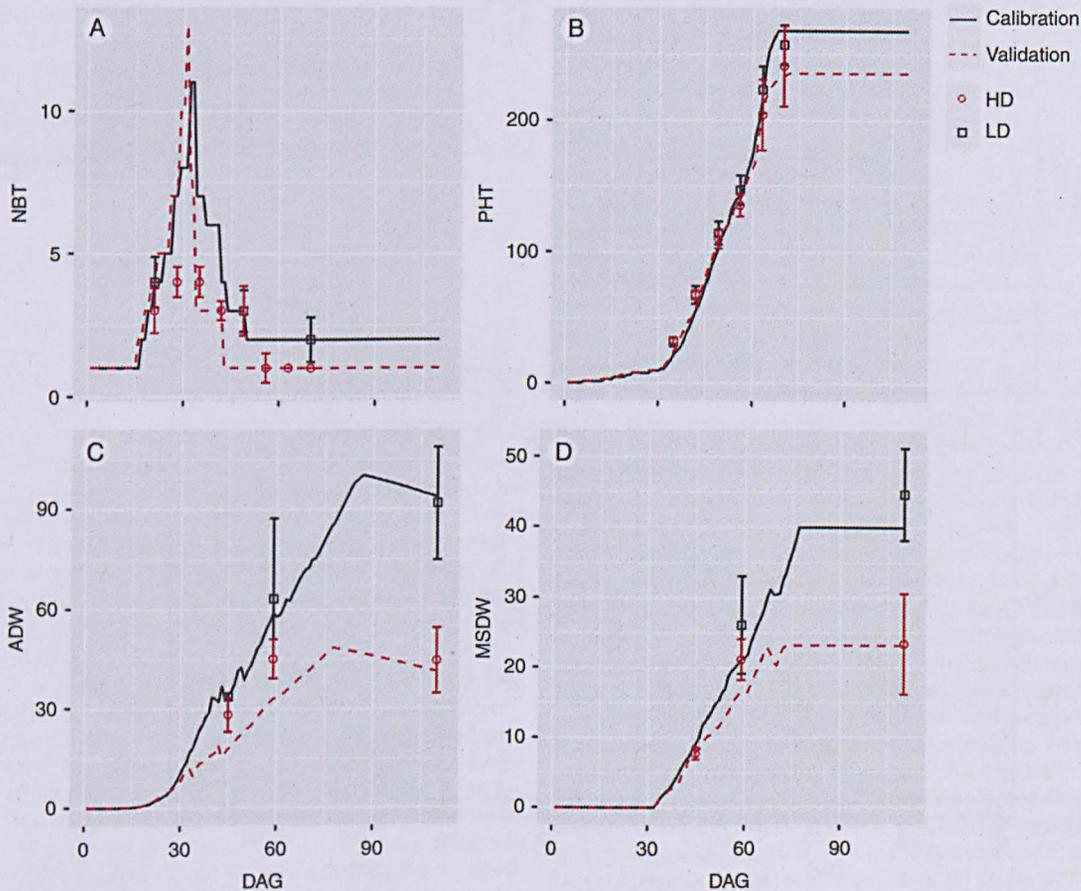


FIG. 3. *Ecomeristem* calibration of low plant density (LD) treatment (20 plants  $m^{-2}$ ) and validation on high plant density (HD) treatment (40 plants  $m^{-2}$ ); example of genotype G6 in the 2016 experiment for four simulated output variables: (A) NBT (tiller number), (B) PHT (plant height, cm), (C) ADW (above-ground dry weight in g), (D) MSDW (main stem dry weight in g). Red and black dots indicate field measurement means with standard error bars. DAG: days after germination.

its cessation and tiller mortality were largely omitted both in crop models and in FSPMs (Evers and Vos, 2013). Crop models generally do not consider tillering *per se* but only bulk LAI dynamics. This is commonly considered as a main limitation with respect to the challenge of using crop models to dissect the genetic and physiological control of complex traits such as yield (Hammer *et al.*, 2010; Kim *et al.*, 2010b). Accordingly, crop models are more and more expected to consider tillering processes, but this is still in its infancy (e.g. in sorghum and rice; Alam *et al.*, 2014; Kumar *et al.*, 2016). In contrast, several 3-D plant models already dealt with tillering processes (Evers and Vos, 2013) but in a way largely disconnected either from the simulation of crop production or from the consideration of mechanistic (explicative, predictive) processes (Abichou *et al.*, 2018). The concept of a plant C supply–demand ratio underlying the genotypic and environmental control of tillering, initially formalized in *Ecomeristem* (Dingkuhn *et al.*, 2006) and experimentally validated for sorghum (Alam *et al.*, 2014; Kim *et al.*, 2010a; b), also appeared relevant in the present study for predicting tillering cessation. However, it failed in predicting tiller mortality, which required the introduction of a hierarchical access of tiller to plant C resources depending on their

age. This rule was made the same for all genotypes, i.e. that the older the tiller (and thus the main stem in the first place) the better its daily C demand is afforded (up to 100% if possible). Accordingly, the simulated tiller mortality only resulted from the inherent level of competition among tillers due to organ size, growth rate and daily plant-available C. This approach enabled us to simulate final tiller number and its contribution to biomass production, but the dynamics of mortality were trickier to simulate precisely. Apical dominance is known to be largely hormonal and to vary not only among species but also among genotypes, although this has not yet been addressed regarding tiller mortality (Müller and Leyser, 2011). It will be interesting, in the follow-up of this study, to introduce a parameter for tuning the level of apical dominance (e.g. the percentage of daily C demand afforded as a priority for an older tiller before testing the C supply to provide to the next tiller), to check whether it can improve the simulation of tiller mortality across a large range of genotypes and finally explore its potential impact on stem biomass production.

The second approach presented in this study introduced a more sophisticated light interception model to deal with its variability among culms within the canopy and in terms of diffuse

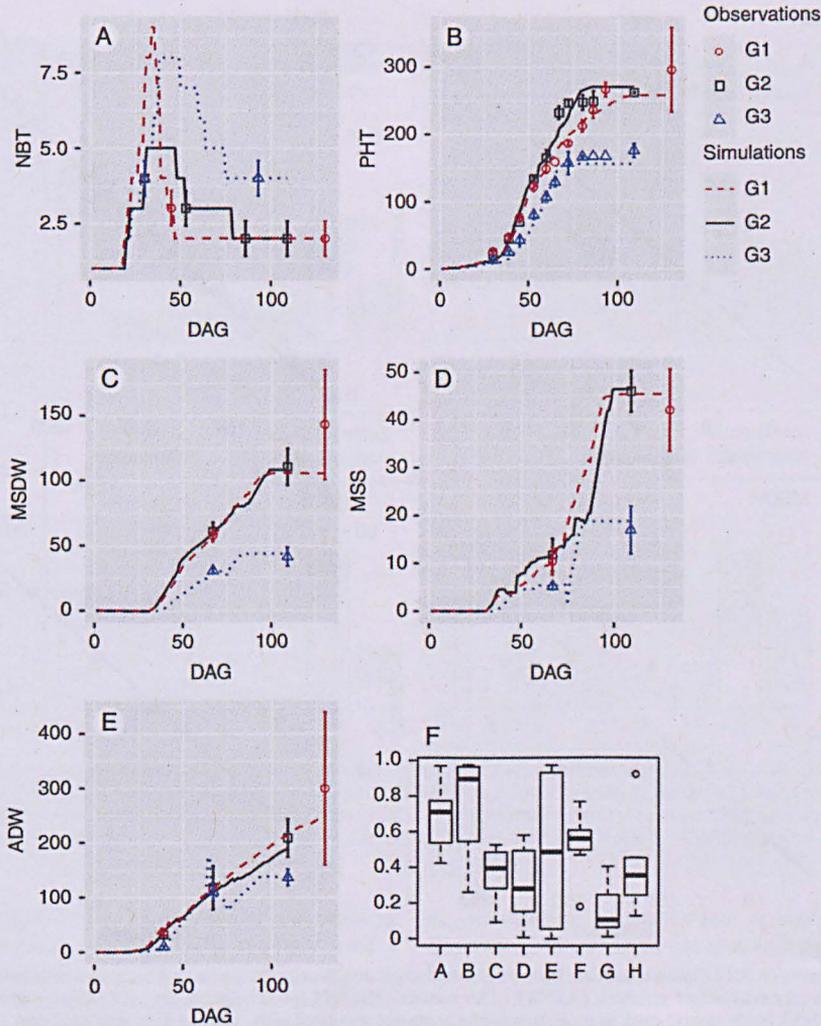


FIG. 4. *Ecomeristem* calibration on 2015 experiment data; example of genotypes G3, G9 and G15 for five simulated model output variables: (A) NBT (tiller number), (B) PHT (plant height in cm), (C) MSDW (main stem dry weight in g), (D) MSS (main stem C stock in g) and (E) ADW (above-ground dry weight in g). Dots indicate field measurements for each genotype with the same colour code as simulations (means with standard error bars). (F) Box plot showing the range of estimated values for seven parameters (A = Epsib, B = lct, C = MGR\_init, D = slope\_LL\_BL\_at\_PI, E = slope\_length\_IN, G = density\_IN2, H = phyllo, detailed in Table 1) and the measured parameter CoefflinINDiam (F). For ease of reading, parameter values were normalized between [0, 1]. DAG: days after germination.

and direct light. Even though it did not strongly contribute to improve the prediction of tiller mortality, it does not minimize the role architectural modelling and FSPMs can play in an ideotyping context, particularly for biomass crops or when dealing with more complex cropping systems (e.g. intercropping, cf. ongoing H2020 project <https://www.remix-intercrops.eu/>).

Stem C storage and partitioning between non-structural and structural carbohydrates was correctly captured by the model. This represents an important feature for a model dedicated to the simulation of phenotypes of a bioenergy crop such as biomass sorghum (Trouche *et al.*, 2014; Xu *et al.*, 2015). Some crop models already paid attention to the partitioning of stem structural and non-structural biomass, in particular for predicting sugarcane yields (Singels and Bezuidenhout, 2002). However, such models were not dedicated to capture the traits underlying G × E at the organ or culm level, which can be a

strong limitation for ideotype design in increasingly fluctuating and extreme climate conditions (Rötter *et al.*, 2015; Wu *et al.*, 2016).

The percentage of dry weight potentially represented by C storage (non-structural biomass) is handled in *Ecomeristem* by an organ-specific parameter fixed to 75 % (internodes) and 40 % (leaves) for all genotypes. The actual daily amount of C supply stored is thus the minimum between the available reservoir in storage organs and the remaining C supply after all demands for structural biomass are satisfied. Considering that this C pool can be mobilized if the daily plant C supply is less than demand, leaf and internode sugar content dynamics depend on the genotype and the environment, and are emerging properties of the model simulations. The fact that this conceptual choice enabled us to simulate correctly stem sugar content is thus quite original. It can be mentioned that another genotypic parameter

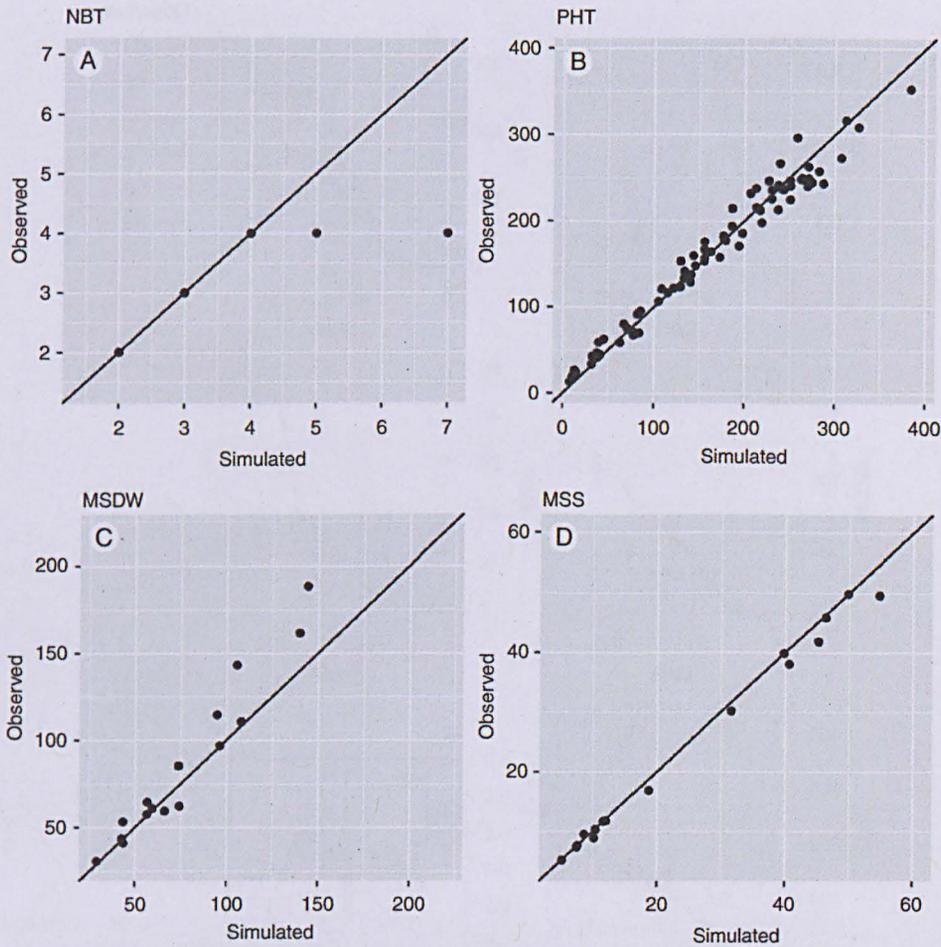


FIG. 5. Validation of *Ecomeristem* on 2014 experimental data for the eight studied genotypes using the parameters estimated with 2015 experimental data (calibration in Fig. 4), for four simulated model output variables: (A) NBT (tiller number), (B) PHT (plant height in cm), (C) MSDW (main stem dry weight in g), (D) MSS (main stem C stock in g). Each sub-plot compares simulated with averaged daily measured data.

related to C storage processes was implemented in the model but not used in this study. This parameter, fixed at 0 here, defines whether and to what extent the daily C storage in the stem is an active sink. The value of this parameter determines the percentage of the available C storage reservoir functioning as an active sink and thus accounted for to compute the daily plant demand. By fixing this parameter to zero in this study, we considered that C storage in internodes was only a passive process, i.e. the result of a spill-over of excess C supply once all the C demands for structural growth were afforded. Whether C storage in sorghum (and sugarcane) internodes is passive (result of a spill-over of C assimilate surplus once C demands for growth are satisfied) or active (dependent on the activity of sucrose synthesis enzymes and transporters; Bihmidine *et al.*, 2016) remains only partially understood. This has strong implications, however, for biomass sorghum improvement as a bioenergy crop. Having implemented such a parameter in *Ecomeristem* will enable evaluation of the impact of this putative process on biomass production and its implication for crop improvement.

**Modelling limitations to be overcome.** The present study only focused on eight biomass sorghum genotypes, and the range of environmental conditions addressed was relatively narrow. In a climate change context and regarding current sorghum cropping environments, it will be essential to extrapolate this study to drought-prone environments and environments where vapour pressure deficit (VPD) fluctuates and can become high. Both drought and high VPD conditions are known to affect organ growth (e.g. in maize, *Chenu et al.*, 2007) and water use (sorghum and maize, *Truong et al.*, 2017). *Ecomeristem* is already equipped with modules to deal with soil water balance and its impact on organ growth, stomatal aperture and C assimilation rate (*Luquet et al.*, 2016). However very little is known about the genotypic sensitivity of internode growth to drought or VPD and whether this sensitivity is proportional between leaves and internodes for a given genotype. Preliminary results in our lab suggest that this is the case. This will have to be further studied and implemented in the model, although it can be expected that sorghum will be less sensitive than maize (*Truong et al.*, 2017).

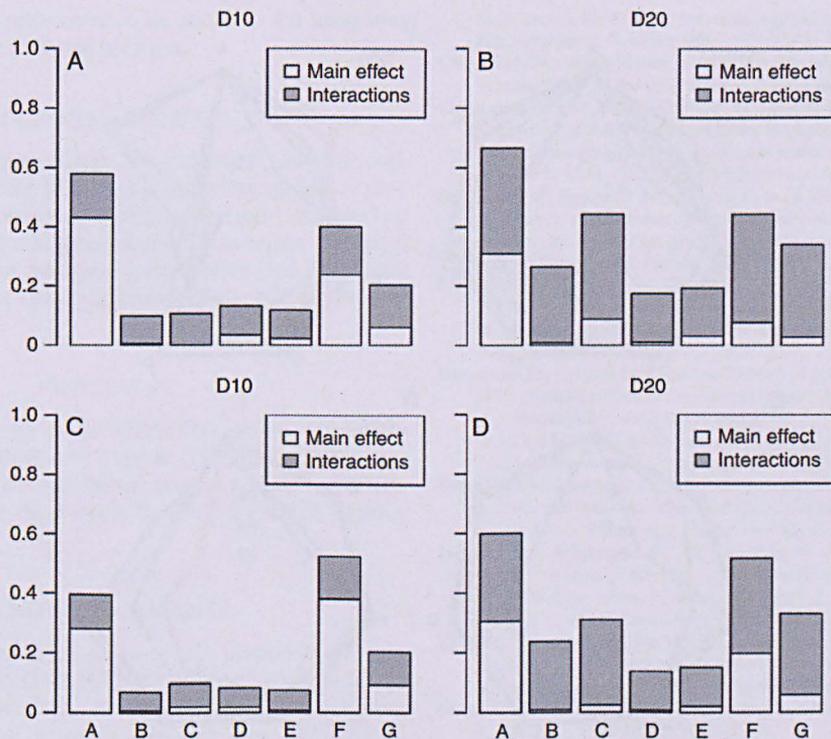


FIG. 6. Sensitivity analysis results showing the main and interaction effects of eight key genotypic parameters on simulated plant stem dry weight (A, B) and plant stem C storage (C, D), at two planting densities [10 (D10: A,C) and 20 (D20: B,D) plants m<sup>-2</sup>] for a standard, theoretical long cycle genotype (final leaf number of 23 on the main stem with development rates similar to G1). Simulation of 130 d after germination (until the grain-filling stage) using 2014 meteorological data. Parameters correspond to A = Epsib, B = Ict, C = MGR\_init, D = slope\_LL\_BL\_at\_PI, E = slope\_length\_IN, F = CoefflinINDiam, G = density\_IN2 (detailed in Table 1).

Another important improvement of the model will concern the thermal time computation. In this study, we only used a base temperature, because the thermal conditions considered were not extreme. However, it will be necessary to use a more precise model considering other cardinal temperatures in studies with more extreme climatic conditions (as in the RIDEV model, Dingkuhn *et al.*, 2017). Finally, as mentioned above, the stem biomass quality was considered here as the partitioning between structural and non-structural carbohydrates. However, the cell wall composition (lignin, cellulose and hemicellulose fractions) and its response to the environment are key features for defining bioenergy crop ideotypes, and more and more is becoming known about the dynamic set-up of the components and their G × E control (Perrier *et al.*, 2017; Luquet *et al.*, 2018). *Ecomeristem* will provide a relevant modelling framework to integrate this kind of knowledge for *in silico* studies. Similarly, the internode thickening (diametric growth) needs to be better represented during internode elongation. Currently a given internode starts elongating with its final diameter, which may affect the precision of the C supply–demand balance in the model (Lacube *et al.*, 2017).

Finally, in a climate change context, the response of a plant to increased atmospheric CO<sub>2</sub> together with other key climatic variables is also crucial (Yin *et al.*, 2016). We have already developed a leaf C assimilation model potentially connected to

*Ecomeristem* to run at the leaf or leaf layer level. This could improve C assimilation computation and ideotype exploration for future climatic scenarios.

#### *Gain in complementarity of ecophysiological models to genetic models in a pre-breeding context*

In this study, a dedicated metaheuristic algorithm was used to estimate conjointly more than ten parameters of *Ecomeristem*. This type of algorithm comes as a powerful tool for model-assisted phenotyping, with a genotype being characterized by a set of model parameter values dissecting a complex trait into elemental process-based traits that are genetically simpler. Model-assisted phenotyping demonstrated its added value 15 years ago (Reymond *et al.*, 2003); however, the use of estimated instead of measured parameters is much less frequent. Indeed, the models used for this purpose generally deal with only one elemental process and few (less than five) parameters underlying plant growth or yield, and do not require metaheuristics (e.g. parameters related to leaf elongation rate vs. grain yield simulation in corn, Chenu *et al.*, 2009). Technow *et al.*, (2015) considered in this context five parameters underlying grain yield in maize, but these parameters remained too complex/integrative to dissect finely the genetic and environmental

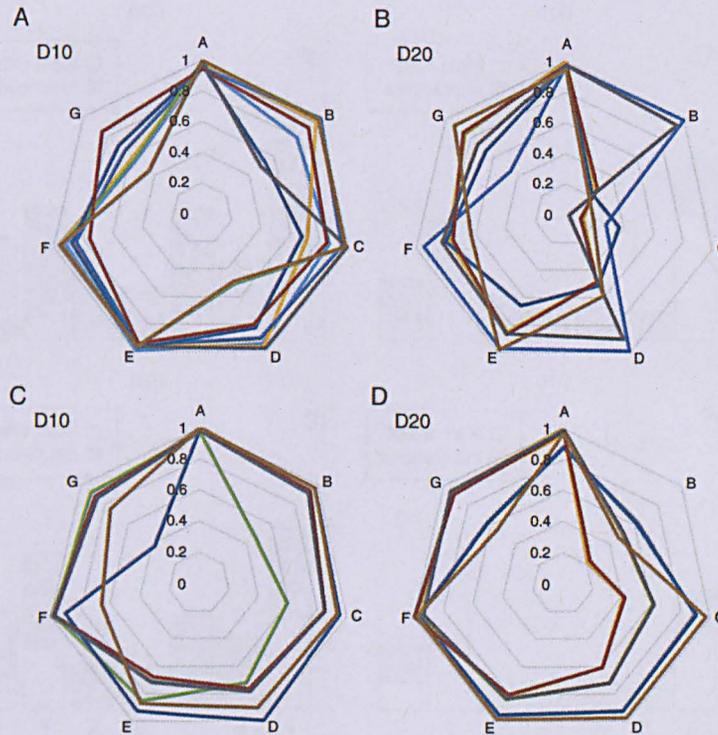


FIG. 7. Radar plots of the eight key genotypic parameter values (detailed in Table 1) met across the ten best ideotypes identified within the simulations performed for the sensitivity analysis (presented in Fig. 6): ideotypes for plant stem dry weight (AB); plant stem C storage (CD), at two planting densities [10 (D10: AC) and 20 (D20: BD) plants  $m^{-2}$ ], for a standard, theoretical long-cycle genotype (final leaf number of 23 on the main stem with development rates similar to G1). Simulation of 130 d after germination (until the grain-filling stage). Parameters correspond to A = Epsib, B = lct, C = MGR\_init, D = slope\_LL\_BL\_at\_PI, E = slope\_length\_IN, F = CoefflinINDiam, G = density\_IN2. For ease of reading, parameter values were normalized between [0, 1].

control of the simulated complex trait. Accordingly, the role ecophysiological modelling can play, combined with appropriate parameter estimation approaches, to dissect the genetic and physiological control of yields is still challenging. The approach presented in this study enabled us, based on a vector of parameters, to capture more exhaustively the genotypic value of a set of traits constituting the elaboration of the whole-plant phenotype. It also enables us to account for the (genotypic) covariation and the trade-offs among these traits, which is essential to explore ideotypes in targeted environments. A key challenge for model-assisted ideotyping will be the coupling with genetic modelling to relate genotypic parameters to genetic information (Yin *et al.*, 2016).

## CONCLUSIONS

In this study, we showed that *Ecomeristem* could be a relevant tool for the model-assisted ideotyping of biomass sorghum. Nevertheless, some methodological considerations that could improve (or set limits to) its applications in a pre-breeding context were not taken into account. First, during parameter estimation, only the total RMSE over all variables was considered, reducing the fitness of the simulation to a single (integrated) criterion. The ideotyping of biomass crops must be multi-criteria because it must consider the component traits of biomass quantity (organ size, development rate, tillering, etc.) and quality (fibres, sugars, lignin, hemicellulose, etc.) and their respective response to the environment. This makes it a multi-criteria

optimization problem, and appropriate algorithms will have to be mobilized in this context (Constantinescu *et al.*, 2016). A second consideration is computation time. Complicating the model to deal with processes of interest for biomass sorghum ideotyping at finer biological and/or temporal scales (e.g. light interception on 3-D plants or C assimilation of individual leaves) will dramatically increase the simulation time (Tremblay and Wallach, 2004). The number of additional parameters implied should be also problematic for estimation algorithms because the phenotypic and numerical space to be explored will be exponentially larger (Constantinescu *et al.*, 2016).

## Perspectives

The simulation experiments carried out with *Ecomeristem* in this study provided insight to define biomass sorghum ideotypes for structural or non-structural biomass production depending on planting density modalities. It was pointed out that, within the range of genetic diversity explored, light conversion efficiency and internode diameters were systematically key drivers of improvement. It was also suggested that sorghum tillering capacity should be further valorized to maintain or even increase biomass yield while using lower planting densities.

More generally, the present study demonstrated the relevance of *Ecomeristem* to capture the genotypic and environmental variability of biomass sorghum phenotypes. This model is currently applied to dissect the genetic and environmental (including water deficit) control of biomass growth within a larger sorghum diversity panel phenotyped within the Phenoarch platform; this will enable genetic

information on model parameters to be added to the integrative approach proposed here to design ideotypes.

#### SUPPLEMENTARY DATA

Supplementary data are available online at <https://academic.oup.com/aob> and consist of the following. Figure S1: example of phytomer and leaf growth phases on sorghum main stem simulated by *EcoMeristem* along thermal time. Figure S2: averaged simulated values over ten ideotypes maximizing either plant stem dry weight or C storage per plant at a planting density of 10 or 20 plants m<sup>-2</sup>.

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