



A model co-ordinating the elongation of all leaves of a sorghum cultivar was applied to both Mediterranean and Sahelian conditions

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Abstract

Sorghum leaf development was analysed at plant level by analysing the time-course of elongation and identifying the beginning and end of the elongation phases of each leaf blade. This was done with destructive and non-destructive measurements in 14 experiments carried out during several growing periods in Southern France and Sahelian Africa. Elongation of each blade was characterized by the succession of a nearly exponential phase and a linear phase. For a given blade and provided that time was expressed in thermal units, initiation, beginning and end of the linear phase, and time-courses of elongation rate were strikingly similar in all experiments, except in environments with a maximum air temperature close to 40 °C and a maximum vapour pressure deficit close to 6 kPa. The relative elongation rate during the exponential phase declined with leaf number from 0.08 to 0.02 °Cd⁻¹, while the duration of this phase increased from 140 to 320 °Cd. By contrast, the absolute elongation rate during the linear phase was nearly constant from leaf 8 onwards. This phase was shorter than the exponential phase regardless of leaf position, but accounted for the largest part of blade length. A strict pattern of leaf development was observed at the whole plant level, whereby dates of elongation events and leaf and ligule appearance, represented on a thermal time scale, were linearly related to phytomer number. This pattern exhibited a simultaneous elongation cessation of the last-formed leaves and a mismatch between real and apparent (from leaf to ligule appearance) elongation duration.

Key words: Blade elongation duration, blade elongation rate, leaf initiation, monocotyledon leaf development, thermal time.

Introduction

In monocotyledons, the expanding region of leaves, where cell division and tissue expansion take place, is located near the leaf insertion point (Schnyder *et al.*, 1987; Paolillo and Sorrells, 1992; Skinner and Nelson, 1994; Ben Haj Salah and Tardieu, 1995). During the first phase of leaf development, this zone increases in length with time, thereby causing an acceleration of leaf elongation rate which is nearly exponential (Gallagher, 1979; Muller *et al.*, 2001). This phase usually ends before the leaf emerges above the whorl. During the second phase, the spatial distribution of relative elongation rate is in steady-state for several days (Schnyder *et al.*, 1990; Bernstein *et al.*, 1995; Muller *et al.*, 2001) resulting in linear elongation of the whole leaf. This implies that the relative elongation rate is essentially constant during the first phase, and that the absolute elongation rate is constant during the second phase. Elongation of each leaf can therefore be characterized by four variables, the relative elongation rate during the first phase and the duration of this phase, and the absolute elongation rate during the second phase and the duration of this phase.

An extension of these results would be to determine the four variables characterizing leaf elongation in all phytomers of the plant, in order to build a model of leaf development at plant level. Elements exist in the literature

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for the determination of rates at different phytomer positions. A reduction in relative elongation rate during the first phase with leaf position in wheat and barley has been observed (Gallagher, 1979). Several authors observed a stability in absolute elongation rate during the second phase in several phytomers in millet, wheat and maize (Ong, 1983b; Baker *et al.*, 1986; Khouja, 1990). By contrast, estimations of the duration of the near-exponential and linear phases are not available in the literature. Leaf elongation duration is often estimated either as the ratio of final length to absolute elongation rate (Hesketh and Warrington, 1989), or as the interval between leaf emergence and ligule appearance (Zur *et al.*, 1989; Stewart and Dwyer, 1994; Bahmani *et al.*, 2000). These methods neglect the long-lasting part of elongation that occurs before the leaf becomes visible and consider that the end of blade elongation occurs at ligule appearance, which is not always the case.

The objective of this study was therefore to establish this whole-plant model of leaf development under non-stressing environmental conditions. This involved co-ordinating the durations of each phase for each phytomer and estimating elongation rates at all phytomer positions. While leaf elongation, when measured non-destructively, corresponds to the cumulative elongation of blade and sheath, this study focused on the blade since light interception relies essentially on blade area only. Because such a model can be useful only if it has some degree of generality, it was decided to establish it under the contrasting locations of Mediterranean and Sahelian conditions, and in the absence of appreciable water deficit. It was also decided to establish it for plants with contrasting number of phytomers brought about by changes in photoperiod.

A condition for such a model to apply across locations is that changes in temperature are taken into account in the approach, because meristem temperature drives leaf elongation in favourable growing conditions. Leaf elongation rate of sorghum leaves located at different positions on the stem respond linearly to meristem temperatures ranging from 13–32 °C (Lafarge *et al.*, 1998), which is consistent with results in other plant species (Dale, 1982; Ben Haj Salah and Tardieu, 1995; Bonhomme, 2000). The duration of leaf expansion relates linearly to the reciprocal of the difference between leaf temperature and the threshold temperature causing cessation of elongation (Granier and Tardieu, 1998; Bonhomme, 2000). These findings can be expressed in a simple way if time is expressed in thermal units, because both rates and durations become independent of temperature, resulting in a temperature-invariant profile of local elongation rate in the leaf (Tardieu *et al.*, 2000). Thermal time was therefore used in this study to analyse time-courses of leaf elongation in a range of environmental conditions.

Materials and methods

Plant material and growing conditions in Bamako

Grain sorghum (*Sorghum bicolor* (L.) Moench) plants were grown in a field in Sotuba (12° 39' N, 07° 56' W, 320 m altitude) near Bamako, Mali. Soil in the upper layers (0–0.2 m) comprised 75% sand, 20% silt and 5% clay, and in the deeper layers (0.2–1 m) 55% sand, 25% silt and 20% clay. It was fertilized with 225 kg of N, P and K ha⁻¹ before sowing and 22.5 kg of N ha⁻¹ at the beginning of stem elongation. Plants belonged to line E-35-1 of Ethiopian origin. Nine growing periods were analysed, with sowing dates on 4 and 30 October 1995 (Exps BA1 and BA3), and 29 March (Exp. BW2), 12 and 21 June (Exps BS1 and BS2), 1, 10 and 16 July, and 10 August 1996 (Exps BS3, BS5, BS6, and BS8) (Table 1). Growing periods from June to September took place during the rainy season, the common growing period of sorghum in the region. Other growing periods were analysed to widen the range of environmental conditions. Plant density was 5.5 m⁻² (0.6 m between rows, 0.3 m between plants in the row). Plants had from 0 to 2 tillers, with a mean number of mature tillers from 0 (Exps BS3 to BS8) to 0.9 (Exp. BW2). Final leaf number per plant ranged from 18 to 31, depending on the growing period (Table 1).

Air temperature and relative humidity were measured with a sensor (HMP35A, Vaisala Oy, Helsinki, Finland) placed in a ventilated cylinder 2 m from the soil above an uncultivated soil area and 5 m away from the border rows. Meristem temperature was measured with 0.4 mm diameter thermocouples inserted in the meristematic zones of three plants per plot. Meristem-to-air vapour pressure deficit was calculated as the difference between saturated pressure of water at meristem temperature minus that at air dew point temperature. Windspeed was measured with a 05103 wind monitor (Young Company, Traverse City, MI, USA). Photosynthetically photon flux density (PPFD) was measured with a sensor (PAR/CBE, Solems, Palaiseau, Essonne, France) that had been calibrated with a Li-Cor cell (Quantum, Li-Cor, Lincoln, Nebraska, USA). All data were stored in a datalogger (CR10, Campbell Scientific, Shepshed, Leicestershire, UK) every minute and averaged every 30 min. In experiments BA and BW, a technical problem did not allow measurements of meristem temperature for long periods, which was estimated from air temperature, air vapour pressure deficit, net radiation and windspeed by using the model of Cellier *et al.* (Cellier *et al.*, 1993). This model gave good estimates of meristem temperature (an average of 0.8 °C was observed between measured and estimated temperature for a period of 70 d in the summertime in Bamako). Rainfall was 94 mm in October 1995, 550 mm from beginning of June to end of September 1996, with 315 mm in Exp. BS1 and 380 mm in Exp. BS5 during leaf development, and 0 mm for the rest of the season. Plants were watered every 2–3 d when necessary, in such a way that soil water potential, measured at 0.5 m with ceramic tensiometers, never declined below –20 kP. Irrigation was 195 mm in Exp. BS1 and 70 mm in Exp. BS5 during leaf development. Photoperiod was calculated as the time during which the sun was located above –6° from the horizontal (Aitken, 1974).

Plant material and growing conditions in Montpellier

Sorghum plants of the same cultivar were grown near Montpellier, France (43° 38' N, 3° 53' E, 50 m altitude) in a deep soil with 45% silt, 40% sand and 15% clay fertilized with 45 and 115 kg of N and P ha⁻¹, respectively. Plants were sown on 17 May, 13 and 27 June and 10 July 1995, and 11 July 1997 (Exps M1 to M5) with the same plant density and spatial

Table 1. Average environmental conditions from seedling emergence to end of leaf development

In the first column, 'M' is for Montpellier, 'BA' for Bamako in Autumn, 'BW' for Bamako in Winter, and 'BS' for Bamako in Summer. In the last two columns, '+' means that LER or LAR were measured. PPFD, photosynthetically active photon flux density; VPD, vapour pressure deficit; LER, leaf elongation rate; LAR, leaf appearance rate.

Experiment	Planting date	Final leaf number	Photoperiod (h)		Daily PPFD (mol m ⁻² d ⁻¹)	Air temperature (°C)			Day air VPD (kPa)		Measurements	
			Seedling emergence	Panicle initiation		Min	Max	Mean	Min	Max	LAR	LER
Summer in Montpellier												
M1	17/05/95	22.8±0.7	16.0	16.3	52.0±10.4	7.6	34.4	22.5	0.2	3.2	+	-
M2	13/06/95	23.3±0.5	16.5	15.7	50.9±9.6	11.1	34.4	23.7	0.5	3.2	+	+
M3	27/06/95	23.3±0.5	16.5	15.2	48.8±9.0	8.4	34.4	23.3	0.5	3.2	+	+
M4	10/07/95		16.2	14.8	45.7±10.4	7.9	34.4	22.7	0.4	3.2	-	+
M5	11/07/97	22.1±0.4	16.2	14.8	41.9±12.4	7.9	33.5	22.5	0.1	2.6	+	+
Dry season in Bamako												
BA1	04/10/95	18.6±0.4	12.7	12.4	44.4±4.5	14.1	35.1	24.3	1.1	3.5	+	-
BA3	30/10/95	18.5±0.5	12.4	12.2	38.8±8.3	10.7	35.1	23.9	1.4	3.6	+	-
BW2	29/03/96	31.0±0.8	13.0	13.6	48.7±9.2	21.2	40.7	30.0	0.8	6.0	+	+
Wet season in Bamako												
BS1	12/06/96	26.8±0.6	13.6	13.5	44.9±10.6	19.9	38.3	26.5	0.3	3.1	+	+
BS2	21/06/96	25.2±0.7	13.6	13.4	44.8±10.6	19.9	35.2	26.1	0.3	2.5	-	+
BS3	01/07/96	23.0±0.5	13.6	13.4	42.6±11.3	19.9	35.1	25.8	0.3	2.1	-	+
BS5	10/07/96	22.5±0.5	13.6	13.3	40.7±12.0	19.9	35.1	25.5	0.1	1.9	+	+
BS6	16/07/96	22.1±0.6	13.5	13.2	39.4±11.5	19.9	35.1	25.4	0.1	1.8	-	+
BS8	10/08/96	19.5±0.4	13.3	13.0	38.4±10.9	19.9	35.1	25.7	0.1	2.0	+	-

arrangement as in Sotuba. Plants had 22–23 leaves, and 0–2 tillers with a mean number of mature tillers of 0.4 (Table 1). Irrigation and collection of meteorological data were performed in the same way as in Sotuba. During leaf development, rainfall was 120 mm and irrigation 210 mm in Exp. M5.

Calculation of thermal time and phenological measurements

Thermal time was calculated by daily integration of meristem temperatures minus a threshold temperature of 10.8 °C (Lafarge *et al.*, 1998). This calculation was used to analyse kinetic developmental events and leaf elongation. Seedling emergence was determined as the day when 50% of plant hypocotyls emerged, i.e. the plant could be seen above the soil surface. The numbers of emerged leaf tips above the whorl and visible ligules were determined every 2–4 d in two area samples with 15 plants each in all experiments (Table 1). Tip and ligule numbers were counted with decimals for better precision as, for tip and ligule, respectively, +0.2 when the leaf tip or ligule had just appeared, +0.6 if the leaf blade or sheath were clearly visible, and +1 when the leaf blade was unfolded or the blade base was separated from the stem. The median leaf number of the 30 plants was kept as the leaf number corresponding to the day under study. Phyllochron was calculated as the time between emergence of two successive leaf tips. Final blade length corresponded to the distance from the leaf ligule to the leaf tip and was measured when the ligule was visible.

Leaf initiation and calculations

The number of leaves initiated on the apex was determined every 2–4 d on three plants in Exps M5, BS1, BS3, BS4, and BS8. Plants were collected from the field at 09.00 h at Bamako and 10.00 h at Montpellier (solar time), and immediately stored at 4 °C for subsequent dissection under a microscope (SZ 6045 TR, Olympus Optical, and MEIJI EMZ, Meiji Techno Co., Tokyo, Japan). A leaf was considered as initiated when its primordium

was visible (about 50 µm long) on the apical meristem with the microscope at magnification × 63.

Leaf elongation measurements and calculations

Lengths of blade (from ligule to tip), sheath (from node to ligule) and internode (between two successive nodes) were measured every second day on all phytomers of three plants in Exp. M5. Plants were sampled from seedling emergence until the last ligule appearance at 10.00 h (solar time), kept at 4 °C and dissected under a microscope (SZ 6045 TR, Olympus Optical, Tokyo, Japan). Phytomer elongation was calculated as the change in cumulative length of the blade, sheath and internode. Elongation was considered to begin on the day when the leaf was initiated on the apex and to end on the day when no more change in length was measured. In addition, blade length was measured from leaf initiation until leaf emergence on phytomers 6, 10 and 14 in Exps BS3 or BS5. Measurements were done in the same way as in Exp. M5.

The change in phytomer length was also measured non-destructively from leaf emergence in all experiments except Exp. M5 (Table 1). Marks were drawn on the blade of ten plants, until the end of leaf elongation, at 18.30 h (solar time) in Bamako and 17.30 h in Montpellier. A fixed horizontal ruler was placed in contact with the blade of leaf 6 at 0.15 m from the soil, allowing the leaf to grow beside it. Marks on the leaf corresponded to the position of the ruler at the time of marking. As the upper leaves grew during stem elongation, the displacement of marks with respect to a fixed altitude would correspond to the sum of blade and stem elongation. So, marks were drawn on leaf *i* at the position of the intersection of the two edges of leaf *i*–1 at the time of marking. When the ligule of leaf *i*–1 had appeared, the mark on leaf *i* corresponded to the position of the ligule of leaf *i*–1. At this time, it was checked that elongation of the sheath of leaf *i*–1 was completed. This method proved reliable since the calculated rates between the destructive and non-destructive methods were not significantly different (Lafarge *et al.*, 1998). The change in phytomer length was

determined as the addition of blade length measured at the time of leaf emergence and of successive distances between marks. It was also checked that the elongation on the main stem did not differ, in a given experiment, between plants with 0, 1 or 2 tillers.

The relative elongation rate (*RER*) was calculated at time *i*, half-time between two consecutive measurements in Exps M5, BS3 or BS5, as the slope of the relationship between the natural logarithm of leaf elongation (*L*) and thermal time:

$$RER = [d(\ln L)/dt]_i \text{ [mm mm}^{-1} \text{ }^\circ\text{Cd}^{-1}] \quad (1)$$

RER was optimized by linear regression on the three coupled values of *L* and *t* corresponding to times *i*−1, *i*, and *i*+1. Maximal *RER* (*RER*_{max}) of a given leaf was calculated as the slope of the relationship between the natural logarithm of leaf elongation and thermal time during the period when the relationship was linear.

Absolute elongation rate (*LER*) was calculated at time *i*, half-time between two consecutive measurements, as the slope of the relationship between leaf elongation (*L*) and thermal time:

$$LER = [dL/dt]_i \text{ [mm }^\circ\text{Cd}^{-1}] \quad (2)$$

LER was optimized by linear regression on the three coupled values of *L* and *t* corresponding to times *i*−1, *i*, and *i*+1. Maximal *LER* (*LER*_{max}) of a given leaf was calculated as the slope of the relationship between leaf elongation and thermal time during the period when the relationship was linear. In the case of non-destructive measurements, thermal time corresponded to the period between tip emergence and two phyllochrons before ligule appearance.

Determination of the end and of the transition between exponential and linear phases of blade elongation

Time of transition between phases of *RER*_{max} and *LER*_{max} was determined graphically from the representation of changes with thermal time in *LER* and *RER* of a given blade collected from leaf initiation in Exps M5, BS3 or BS5. Time could be obtained by determining either the starting date of the period of *LER*_{max} or the finishing date of the period of *RER*_{max}. To minimize experimental errors, time of transition was identified as the abscissa of the intersection point of *LER* and *RER* dynamics, when normalized. The end of blade elongation was determined as the abscissa of the intersection point between final blade length and the regression line which was applied on the linear elongation phase, either of the blade or of the phytomer. Such analysis was done on measurements obtained in Exps M5 and BS. In Exps BS, measurements of the initial blade length at the time of leaf emergence associated with the non-destructive leaf elongation measurements provided an appreciable number of data related to the end of blade elongation, in addition to those obtained from destructive measurements.

Results

Temperature, photoperiod and final leaf number were appreciably variable in the set of experiments

Photoperiod at seedling emergence ranged from 12.4–13.6 h in Bamako and from 16.0–16.5 h in Montpellier (Table 1). In Bamako, the photoperiod from emergence to panicle initiation increased 0.6 h in Exp. BW2 and decreased slowly in the other experiments (0.1–0.3 h), while in Montpellier this decrease

was more rapid (down to 1.5 h). These changes in photoperiod were associated with large differences in leaf number per plant between experiments in Bamako (18.5–31.0) and with small differences in Montpellier (22.1–23.3). Daily photosynthetic photon flux density (PPFD), averaged during the period of leaf development, ranged from 38–49 mol m^{−2} d^{−1} in Bamako and from 42–52 mol m^{−2} d^{−1} in Montpellier (Table 1). A large day-to-day variability was observed in both locations (Fig. 1a, d, g). Appreciable differences in air temperature were observed between experiments, from 22–24 °C in Montpellier, to 25–27 °C in Bamako during the wet season, and up to 30 °C in Bamako during the hot dry season (Table 1; Fig. 1b, e, h). Night temperature was appreciably lower in Montpellier (from 8–11 °C) than in Bamako during the rainy season (20 °C). Air vapour pressure deficits were similar (0.1–3 kPa) in Montpellier and Bamako during the wet season, but were due to different combinations of temperature and dew-point

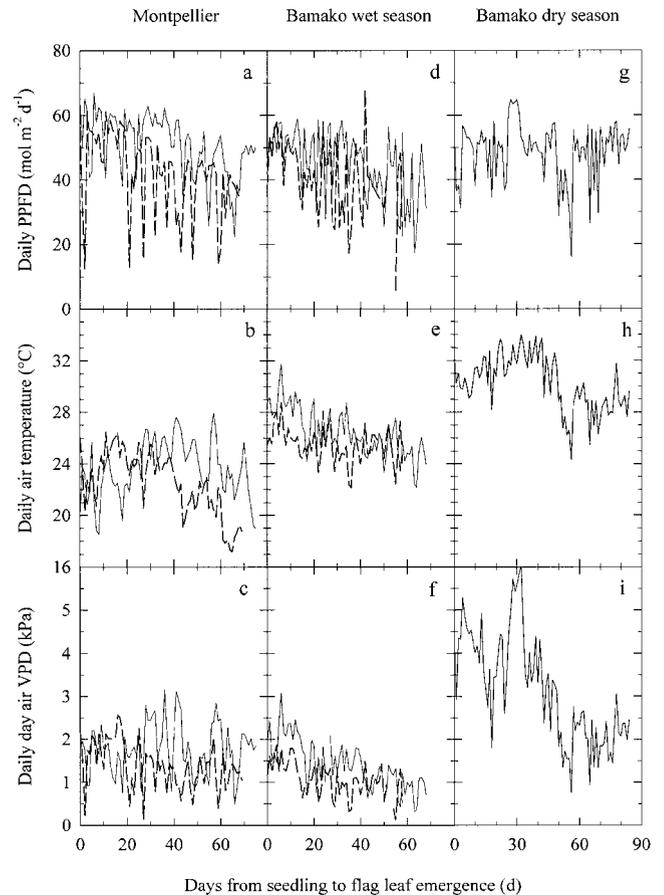


Fig. 1. Change with time in daily photosynthetic photon flux density (PPFD; a, d, g), daily air temperature (b, e, h) and daily day air vapour pressure deficit (VPD; c, f, i) in Montpellier (a–c), in Bamako during the wet season (d–f) and in Bamako during the dry season (g–i) from seedling emergence to flag leaf emergence. (a–c) Thin solid line for Exp. M2; thick dash line for Exp. M5; (d–f) thin solid line for Exp. BS1; thick dash line for Exp. BS5; (g–i) Exp. BW2.

temperature (Table 1; Figs 1c, f). It was much higher in Bamako during the hot dry season, up to 6 kPa (Table 1; Fig. 1i).

Elongation of blade, sheath and internode carried by phytomer 10 had similar time-courses and rates in Montpellier and Bamako

If expressed in thermal time, time-courses of elongation of the blade, sheath and internode carried by phytomer

10 were similar in two experiments in Bamako and Montpellier (Exps M5 and BS3, Fig. 2). This was not the case if time was expressed in calendar time (not shown). Blade elongation lasted 300 °Cd from leaf initiation to the end of elongation (Fig. 2a). The linear elongation of the sheath began 100 °Cd after that of the blade, followed by the onset of internode elongation. Blade elongation was nearly exponential with respect to thermal time for 150 °Cd until the leaf reached 100 mm (linear increase in logarithmic scale, Fig. 2b, constant relative elongation

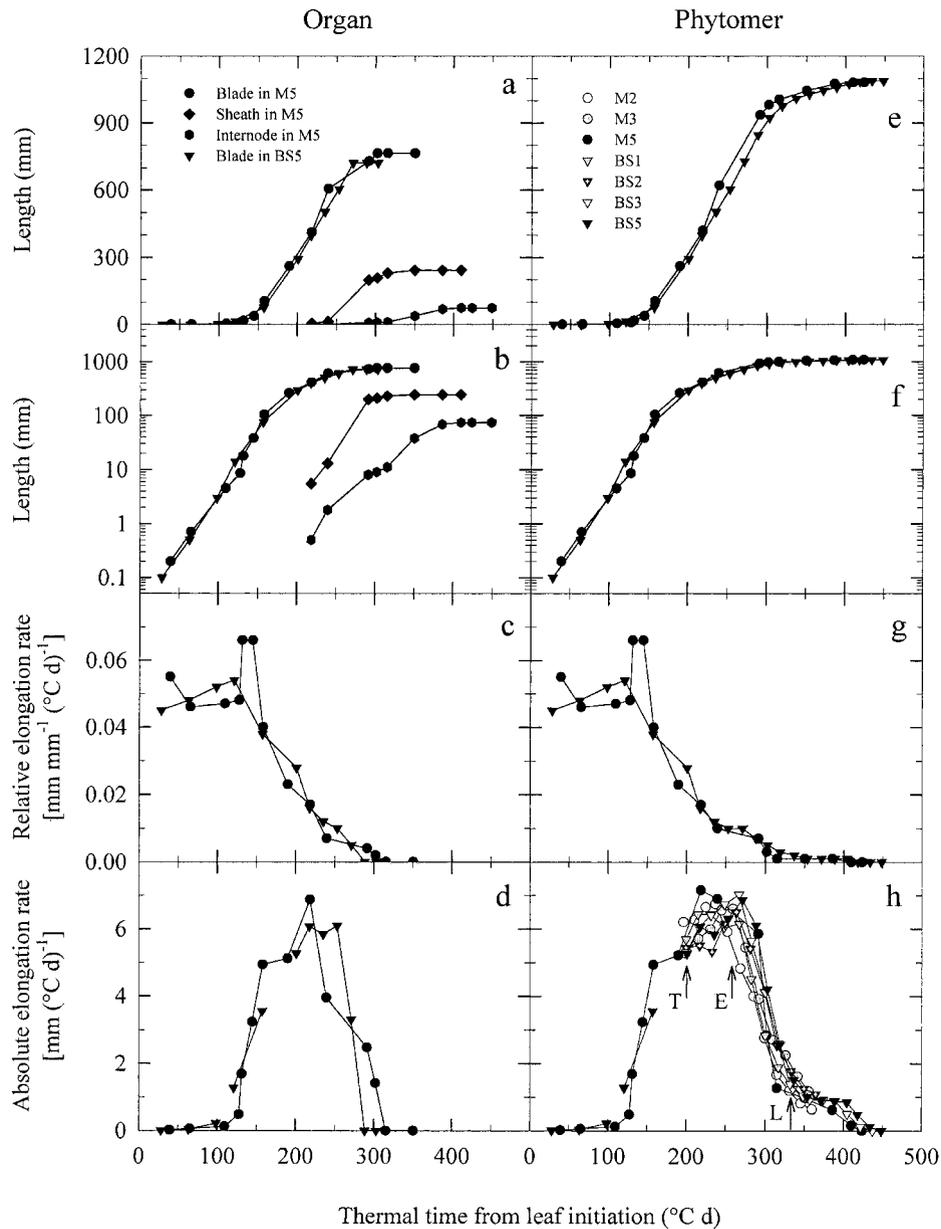


Fig. 2. Time-courses of the lengths of blade, sheath and internode of leaf 10 (a, b), of relative elongation rate (c) and of absolute elongation rate (d) in experiments in Montpellier or Bamako (Exps M5 and BS5). Corresponding values for the whole leaf are presented in (e) to (h). In (h), seven experiments are presented, three in Montpellier (Exps M2, M3 and M5) and four in Bamako (Exps BS1, BS2, BS3, and BS5). T, tip emergence; E, end of blade elongation; L, ligule appearance.

rate at about $0.05 \text{ mm mm}^{-1} \text{ }^\circ\text{Cd}^{-1}$, Fig. 2c). It became linear afterwards until almost the end of elongation (Fig. 2a), at $6 \text{ mm }^\circ\text{Cd}^{-1}$ (Fig. 2d). Two consecutive phases, exponential and linear, also described sheath and internode elongations.

The accumulated elongation of the blade, sheath and internode measured in the same experiments exhibited two distinct phases, one nearly exponential with near-constant relative elongation rate (Fig. 2f, g) and one nearly linear until the end of sheath elongation, before the beginning of rapid internode elongation (Fig. 2e). The absolute elongation rate was stable for a longer period than when the blade only was considered, because sheath elongation accelerated when blade elongation decelerated (Fig. 2h). A transient period of stable rate at $1 \text{ mm }^\circ\text{Cd}^{-1}$, corresponding to rapid internode elongation, was observed between 350 and 400 $^\circ\text{Cd}$. The time-courses of accumulated elongation presented above were very similar to those observed in five experiments where leaf elongation rate was measured non-destructively after leaf emergence (Fig. 2h). Overall, these results suggest that the development of phytomer 10 followed a pattern which was common to all experiments, provided that it was expressed in thermal units. This pattern involved both the duration of the developmental phases and the rates of elongation observed at a given thermal time.

Time-courses of blade elongation differed between phytomers 6, 14 and 22 in Montpellier and Bamako

The time-courses of phytomers 6, 14 and 22 were similar for experiments in Montpellier and Bamako when expressed in thermal units, but markedly differed between leaf positions (Fig. 3). Two phases, exponential and linear, described the blade elongation in the three cases (Fig. 3a, b), but with clear differences in duration of each phase and in elongation rate between leaf positions. The linear phase was three times shorter for blade 6 than for blade 14 and nearly non-existent in blade 22. The relative elongation rate observed during the exponential phase decreased with leaf position, from 0.082 to $0.024 \text{ mm mm}^{-1} \text{ }^\circ\text{Cd}^{-1}$ from leaf 5 to leaf 22 (Figs 3c, 4a). Conversely, the absolute elongation rate observed during the linear phase did not change appreciably from leaf 8 to leaf 20 (Figs 3d, 4b). The latter were common, at a given leaf position, to nine experiments in Montpellier and Bamako (Fig. 4b).

Leaf initiation, the transition between the exponential and linear phases and the end of blade elongation of each leaf occurred at a given thermal time in all experiments

The change with time in the number of leaves initiated on the apex was remarkably stable and similar in

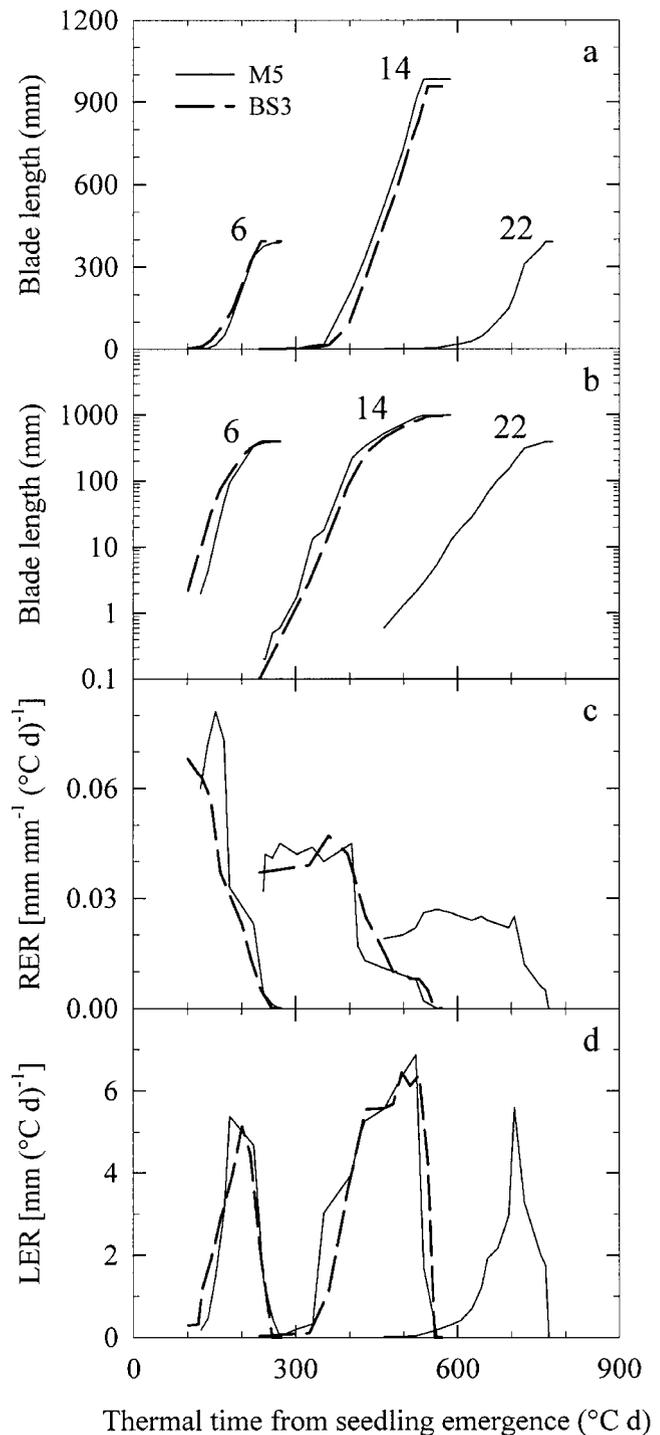


Fig. 3. Comparison of time-courses in leaves 6, 14 and 22. Length of leaf blade (a, b), of blade relative elongation rate (c) and of blade absolute elongation rate (d) in Montpellier and Bamako (Exps M5 and BS3).

experiments in Montpellier (Exp. M5) and Bamako (Exps BS) when expressed per unit thermal time (Fig. 5a). Corresponding relationships did not differ significantly between experiments, so a common linear regression was fitted ($r^2 = 0.99$).

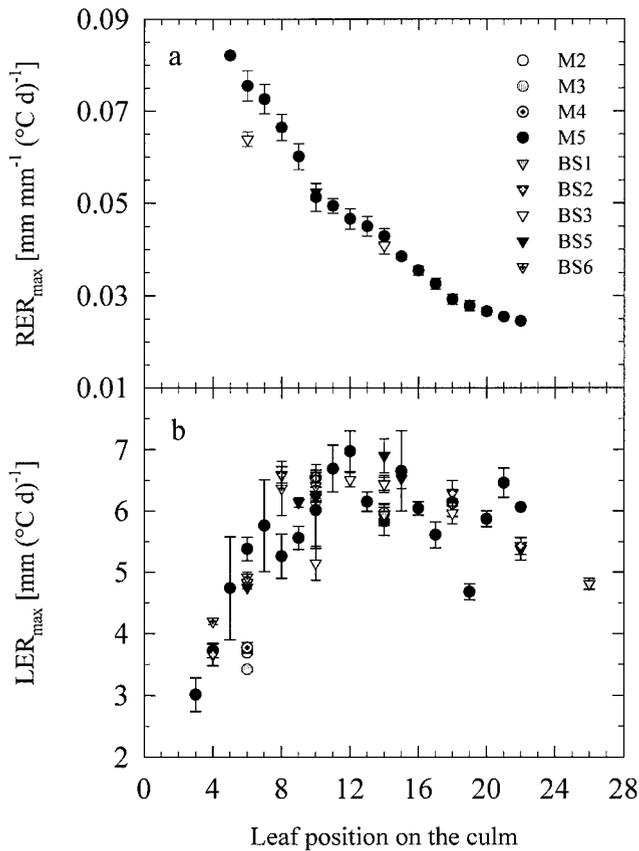


Fig. 4. Relationship between the position of the leaf on the stem and maximal relative elongation rate (RER_{max} , a), or maximal absolute elongation rate (LER_{max} , b) for leaves 3 to 26, measured in four experiments in Montpellier (Exps M2, M3, M4, and M5) and five in Bamako (Exps BS1, BS2, BS3, BS5, and BS6). Vertical lines represent the standard error of the linear regression fitted on data measured during the exponential (linear in a logarithm length scale) and linear elongation phase.

The transition between the exponential and linear phases of blade elongation also occurred in a regular way (Fig. 5b), although a shift in slope occurred around the transition in leaf 13, i.e. when the last leaf was initiated on the apex. The first slope was similar to that of leaf initiation, so the duration of the exponential phase was similar (140–150 °Cd) for leaves 6 to 13 (Fig. 6a). For leaves 13 onwards, the slope was less than that of leaf initiation (Fig. 5b), so the duration of the exponential phase increased with leaf number until 320 °Cd for leaf 22. The duration of the exponential phase determined from data collected in Bamako were similar to those of Montpellier, although values were slightly higher for leaves 6 and 10 (Fig. 6a).

The end of blade elongation was again well co-ordinated in all leaves. The number of leaves whose blade elongation was complete was in close linear relationship with thermal time, with similar values in Montpellier and Bamako, except for the last leaves (Fig. 5c). It is noteworthy that, in Exp. M5, the blades

of the last four leaves stopped elongating almost simultaneously. Except for these leaves, a linear relationship accounted for the cessation of blade elongation ($r^2=0.99$). In Montpellier, the duration of the linear phase increased with leaf position in the first 13 leaves, was nearly stable at 130 °Cd for leaves 13 to 19, and decreased strongly in the last four leaves, to 25 °Cd for leaf 22 (Fig. 6b). The duration of the linear phase in Bamako were similar to those in Montpellier, although values were slightly higher for leaf 6 and lower for leaf 10. In all leaves, the duration of the linear phase was appreciably shorter than that of the exponential phase (Fig. 6a, b). However, the pattern of final blade length approximately followed that of the duration of the linear phase and was completely independent of the duration of the exponential phase (Fig. 6c).

Tip emergence and ligule appearance of a leaf were not associated with either transition or end of elongation

The events of leaf development presented above were compared with those currently observed with non-destructive measurements, namely leaf tip emergence above the whorl and ligule appearance (Fig. 5d, e). The latter events also occurred at a given thermal time in all experiments in Montpellier and Bamako and were linearly related to thermal time. In all growing periods, the ligule appearance rate was higher for the last leaves, with a shift in slope which depended on leaf number. Figure 5f suggests that leaf emergence and ligule appearance did not coincide with either transition or the end of elongation. In all leaves, tip emergence occurred after the end of exponential phase, so elongation was already linear when the leaf became visible, and ligule appearance was observed after the end of elongation. The elongation of blades 21 and 22 in Exp. M5 was already finished when the corresponding tips emerged from the whorl.

High air temperature and vapour pressure deficit conditions had a detrimental effect on leaf elongation and emergence rates

The time-courses and rates presented above did not apply to experiments occurring during the hot dry season in Bamako, where both air temperature and vapour pressure deficit were appreciably higher than in the other conditions (Table 1; Fig. 1). In Exp. BW2, elongation rate of leaf 10 measured from tip emergence departed appreciably from the general pattern observed Fig. 2h (Fig. 7a) and tip emergence rate was appreciably lower than the linear regression fitted Fig. 5d (Fig. 7b).

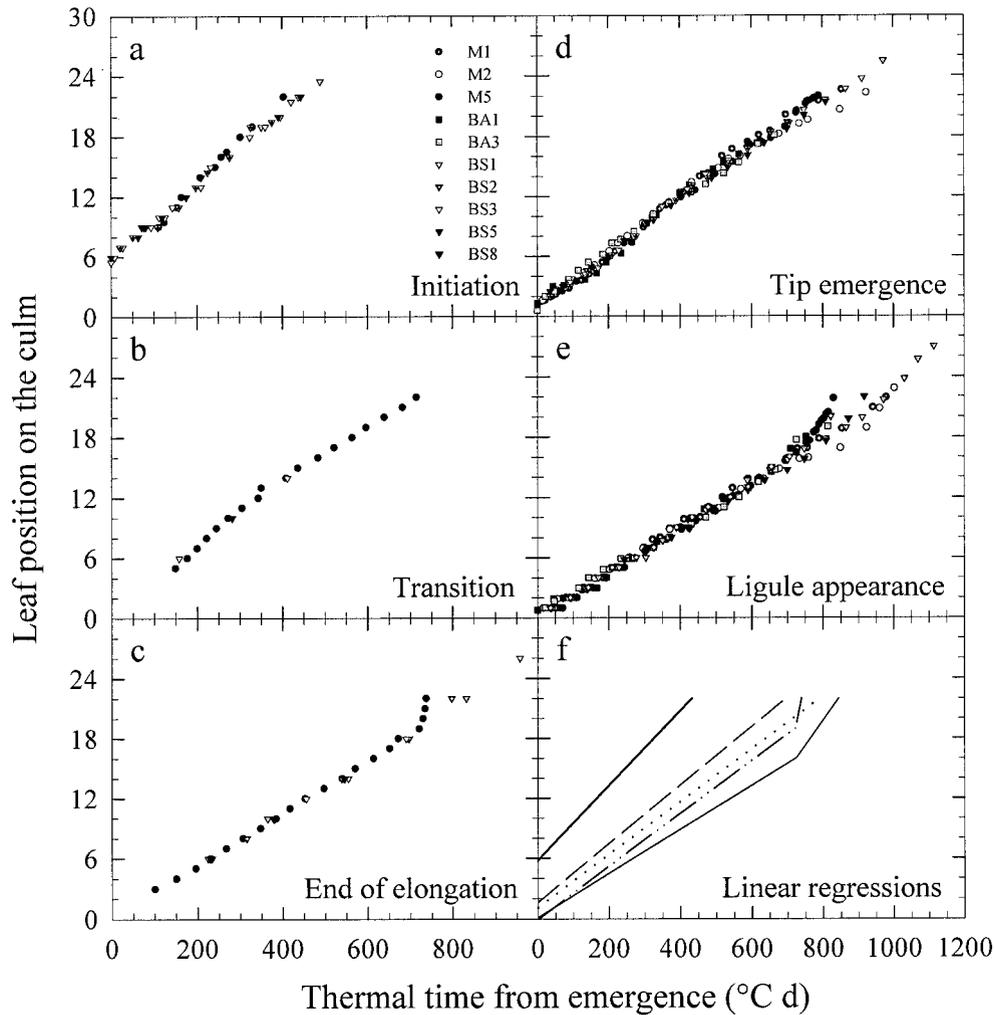


Fig. 5. Time-courses of leaf initiation (a), transition between exponential and linear phases (b), end of leaf elongation (c), leaf tip emergence (d), and leaf ligule appearance (e). Data of blade initiation and elongation originate from Exp. M5 in Montpellier and five experiments in Bamako (Exps BS1, BS2, BS3, BS5, and BS8). Data of tip emergence and ligule appearance originate from three experiments in Montpellier (Exps M1, M2 and M5) and five in Bamako (Exps BA1, BA3, BS1, BS5 and BS8). Data from (a) to (e) are synthesized in (f) for a final leaf number fixed at 22: thick solid line for leaf initiation, dash line for transition, dotted line for tip emergence, dash-dot-dot line for end of elongation, and thin solid line for ligule appearance. Regression lines (with Nb for leaf position on the stem and TT for thermal time): from (a) $Nb = 0.038TT + 5.76$; from (b) $Nb = 0.029TT + 1.63$; from (c) $Nb1 = 0.026TT - 0.09$ and $Nb2 = 0.18TT - 107.9$; from (d) $Nb = 0.026TT + 1.06$; from (e) $Nb1 = 0.022TT + 0.13$ and $Nb2 = 0.05TT - 20.8$.

Discussion

The timing of leaf initiation, the transition between the exponential and linear elongation phases and the end of elongation were remarkably similar in field experiments at either Bamako or Montpellier, irrespective of whether the plants had different leaf numbers. Rates of blade elongation, leaf emergence and ligule appearance were also remarkably stable under all these conditions. These findings support the position that leaf development at a given phytomer is strictly defined for a given genotype and can be expressed in a unique way provided that time is expressed in thermal units calculated from meristem temperature. Due to its robust nature,

this time-course of leaf development can therefore be considered as a model of development applicable to the large range of conditions and can probably be extrapolated to other conditions in the absence of appreciable stresses. Photoperiod, soil type and all the other factors which differentiate a semi-arid from a Mediterranean environment had no appreciable influence on the time-course of leaf development. In particular, leaf initiation was linear with thermal time, as also observed on maize (Warrington and Kanemasu, 1983) and millet (Ong, 1983a). In contrast to previous results, it was not affected by variations in photoperiod from 13 to 16 h (Baker and Gallagher, 1983), in temperature from 13 to 38 °C (Hunt and Chapleau, 1986) and in plant age (Zur *et al.*, 1989).

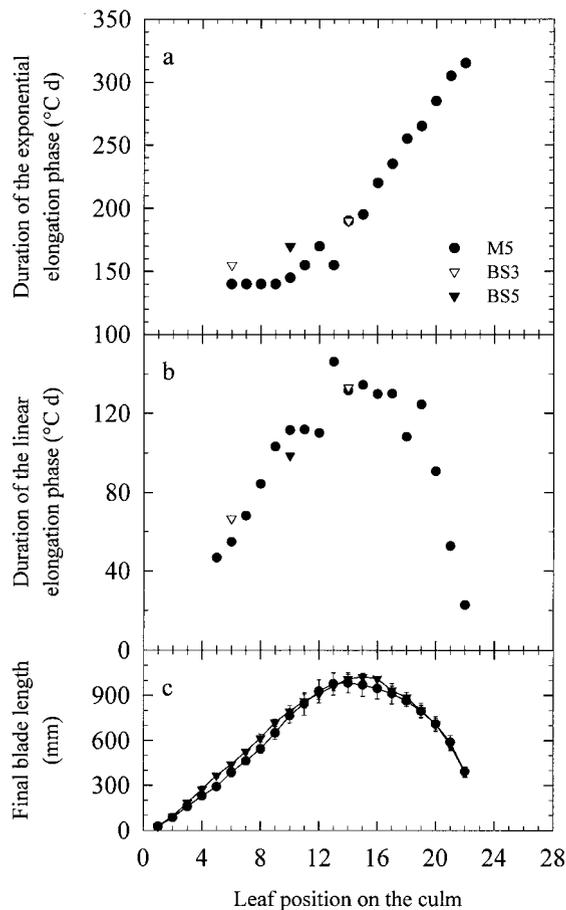


Fig. 6. Relationship between position of the leaf and duration of the exponential elongation phase (a), duration of the linear phase (b) for leaves 5 to 22 and final blade length (c) for leaves 1 to 22, measured in Montpellier and Bamako (Exps M5, BS3 and BS5). The duration of the exponential phase corresponds to the duration between leaf initiation and transition. The duration of the linear phase corresponds to the duration between transition and end of elongation. Final blade length was measured from ligule to leaf tip.

Therefore, in the experiments presented here, which were carried out in the absence of water or nutrient deficits, the time-course of leaf development essentially depended on organ temperature.

However, this model did not apply when vapour pressure deficit reached very high values, such as those observed in Bamako during a season when sorghum is not usually grown. The lower leaf elongation rates which were measured during the hot dry season may be due, either to detrimental effects on plant metabolism caused by high temperatures (close to 40 °C), or to direct effects on elongation caused by high vapour pressure deficits (close to 6 kPa). This was argued earlier for sorghum (Lafarge *et al.*, 1998) and observed in millet (Squire and Ong, 1983) and maize (Ben Haj Salah and Tardieu, 1997). Even in well-watered soils, a high evaporative demand causes a decrease in maize leaf elongation rate, probably mediated via a hydraulic signal.

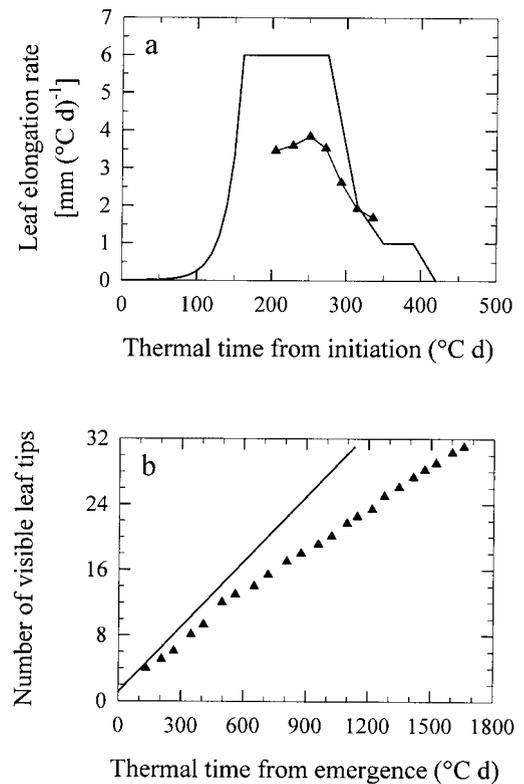


Fig. 7. Time-courses of leaf elongation rate (a) and number of visible leaf tips (b). (a) The solid line represents the tendency from Fig. 2h, and triangles represent data measured in Bamako with high temperature and vapour pressure deficit (Exp. BW2). (b) The solid line represents the tendency from Fig. 5d and triangles represent data measured in Exp. BW2.

The differences in final blade length between phytomers, represented by a bell-shaped curve associated with phytomer position, were essentially linked to differences in duration of the linear elongation phase rather than to differences in blade absolute elongation rate (as suggested by Van Esbroeck *et al.*, 1997). (a) Relative elongation rate and duration of the exponential phase were inversely related to leaf position, so that leaf length was approximately similar in all phytomers at the end of the exponential phase. The reality of the exponential phase was recently questioned earlier (Muller *et al.*, 2001) in maize, because relative elongation rate was never constant during early development. The precision of measurements taken here does not allow discussion of this point. However, near stability was observed over long periods so the concept of an exponential elongation phase can be used in modelling for sorghum. (b) Absolute elongation rate was approximately stable with leaf position from leaf 8 onwards. This stability for upper leaves has also been observed in millet (Ong, 1983b) and wheat (Baker *et al.*, 1986; Bos and Neuteboom, 1998). (c) The duration of the linear phase decreased with leaf position in the last-formed leaves, due to a simultaneous cessation of

elongation. This is in agreement with the marked reduction of the final blade length of these leaves, whereas their elongation rates were similar to those of leaves located at the mid-position of the stem. As in the case of grass species (Durand *et al.*, 1999) and maize (Muller *et al.*, 2001), and regardless of leaf position, the linear phase determined here was systematically shorter than the exponential phase. Its duration varied from 5% to 45% of total blade elongation according to leaf position, but, as also reported previously (Gallagher, 1979), it contributed to the major part of the final blade length, i.e. between 77% and 87% for leaves 6 to 18 (data not shown).

It is important to note that easily observable events such as leaf tip emergence above the whorl and ligule appearance, observed from non-destructive measurements and commonly reported in the literature, did not match the transitional events of blade elongation. Tip emergence happened for most leaves between the beginning of the linear phase and the end of elongation, even after the end of blade elongation for the last-formed leaves. Gallagher also observed that the linear phase started before leaf tip emergence on barley (Gallagher, 1979). Ligule appearance always happened after the end of blade elongation, up to 100 °Cd later for some leaves. Therefore, the duration of apparent elongation (from tip emergence to the ligule appearance) strongly differed from the duration of real elongation (from initiation or transition to the end of elongation) for all leaf positions. In addition, visible elongation rate did not correspond to the effective elongation rate. It is therefore inappropriate to determine the duration of blade elongation (Bos and Neuteboom, 1998; Bahmani *et al.*, 2000) or of blade area expansion (Zur *et al.*, 1989; Stewart and Dwyer, 1994) from non-destructive observation. It is also inappropriate to calculate the duration of elongation by dividing the final blade length by the elongation rate corresponding to the visible phase of elongation (Hesketh and Warrington, 1989).

The co-ordination of leaf blade development presented here, which is based on common linear regressions applying to a range of experimental conditions, was elaborated without the need of specific parameters for each individual leaf or location. These regressions allow the calculation of leaf length under non-stressing conditions, with a lesser role for empirical relationships than most models of leaf development previously reported (Maas, 1993; Hammer and Muchow, 1994; Kaitaniemi *et al.*, 1999). They could easily be included into a computer-generated 3-dimensional representation of virtual plants (Fournier and Andrieu, 1998). Such representations use temperature and genetic characteristics as inputs to simulate the dynamics of leaf area development of each individual leaf of a genotype in a given environmental scenario. A vertical reading of Fig. 5f

indicates the number and position of leaves in a given phase. For instance, at 100 °Cd, the exponential phase of blade elongation concerned five leaves while the linear phase concerned two leaves. A horizontal reading indicates the duration and the position of the exponential and linear phases of each leaf blade. As an example, the blade of leaf 18 elongated from 320 to 680 °Cd after emergence, exponentially during 230 °Cd and linearly during 130 °Cd. This knowledge makes it possible to estimate the leaf and phase of elongation which would be affected by transient stress conditions.

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