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# Distinct controls of leaf widening and elongation by light and evaporative demand in maize

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Controls of leaf elongation and widening

Summary statement

Whilst an appreciable part of the variance of leaf area in the field is explained by leaf width, only few papers analysed separately the effects of environmental conditions on leaf widening and elongation. Here we show that light and evaporative demand have distinct controls on leaf elongation and widening, with very low genetic correlations. The resulting equations allowed better predictions of leaf length, width and final leaf area in contrasted environments over Europe.

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## **Abstract**

Leaf expansion depends on both carbon and water availabilities. In cereals, most of experimental effort has focused on leaf elongation, with essentially hydraulic effects. We have tested if evaporative demand and light could have distinct effects on leaf elongation and widening, and if short term effects could translate into final leaf dimensions. For that, we have monitored leaf widening and elongation in a field experiment with temporary shading, and in a platform experiment with 15-min temporal resolution and contrasting evaporative demands. Leaf widening showed a strong (positive) sensitivity to whole-plant intercepted light and no response to evaporative demand. Leaf elongation was (negatively) sensitive to evaporative demand, without effect of intercepted light *per se*. We have successfully tested resulting equations to predict leaf length and width in an external dataset of 15 field and 6 platform experiments. These effects also applied to a panel of 251 maize hybrids. Leaf length and width presented quantitative trait loci (QTLs) whose allelic effects largely differed between both dimensions but were consistent in the field and the platform, with high QTLxEnvironment interaction. It is therefore worthwhile to identify the genetic and environmental controls of leaf width and leaf length for prediction of plant leaf area.

## **Key words**

Evaporative demand, VPD, light, leaf length, leaf width, leaf elongation, leaf widening, GWAS, QTL.

## Introduction

Leaf area affects the amount of light intercepted by plants, but also their transpiration. In case of drought or heat constraints, reducing leaf area reduces photosynthesis but allows plants to save water for later stages of the crop cycle without risking the over-heating that often accompanies stomatal closure (Guilioni *et al.*, 2008). The understanding of genetic and environmental controls of leaf expansion is therefore central for assessing the trade-off between transpiration and carbon assimilation. For example, a comparison of crop models has shown that the quality of yield prediction by crop models is to a large extent linked to the quality of the modelling of plant leaf area (Martre *et al.*, 2015).

Light and water affect leaf expansion in both monocotyledonous and dicotyledonous species. In the latter, in which leaves expand in two dimensions, light and water affect leaf expansion with markedly different periods of sensitivity within a leaf (Granier & Tardieu, 1999a, b). Young expanding leaves are affected by light through carbon starvation, whilst older expanding leaves are more affected by water via hydraulic limitations (Pantin, Simonneau & Muller, 2012, Pantin *et al.*, 2011). In monocotyledonous leaves, which essentially grow in one dimension, leaf elongation responds to soil water deficit, evaporative demand and temperature in maize, wheat and rice (Ben Haj Salah & Tardieu, 1997, Mahdid *et al.*, 2011, Parent *et al.*, 2010). Light has no direct effect on leaf length (Ben Haj Salah & Tardieu, 1996, Bos, Tijani-Eniola & Struik, 2000), whereas it affects leaf width (Bos, Tijani-Eniola & Struik, 2000, Sonohat & Bonhomme, 1998). However, the effect of light on leaf growth of monocotyledons via changes in leaf width has been the object of few studies. For example, entering "leaf elongation" and "leaf widening" in *The Web of Science*<sup>®</sup> (<https://webofknowledge.com/>) results in 3203 and 63 hits, respectively.

The hypothesis of separate controls for leaf elongation and widening of monocotyledons leaves is supported by several observations. First, final leaf width and length are largely uncorrelated in biparental populations (Wei *et al.*, 2016, Yang C. *et al.*, 2016) resulting in few overlapping QTLs in maize (Tian *et al.*, 2011) and wheat (Yang DL. *et al.*, 2016). Second, leaf elongation and widening occur at different places in the leaf meristematic zone (Maurice, Gastal & Durand, 1997, Muller *et al.*, 2007). Third, environmental variables that control length and width may differ. Differential effects on leaf length and leaf width have been observed in response to plant density in maize (Sonohat & Bonhomme, 1998). Furthermore, leaf width variations have been shown to closely match the decrease in light interception per plant with plant density (Fournier, Andrieu & Sohbi, 2001), whereas leaf length may respond positively or negatively to increased density depending on

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leaf rank (Song *et al.*, 2016, Sonohat & Bonhomme, 1998). This suggests independent effects of light and water limitations.

The above paragraphs suggest a need for the reassessment of the respective roles of carbon and water supplies on leaf expansion in monocotyledons, by distinguishing their respective effects on leaf elongation and widening. This may have a large impact on (i) the modelling of environmental effects, because the response of leaf expansion would not be the consequence of a unique sensitivity to environmental conditions but the combination of the sensitivities of leaf widening and elongation; (ii) in the simulation of the genetic variability of plant leaf area, because leaf length and width may have distinct genetic controls and their sensitivities to light or water may largely differ independently in panels of genotypes.

In this study, we have first identified, via a specific experiment, the phenological phases during which leaf widening and elongation occur. We have then analysed the dynamic responses of leaf widening and elongation to light and evaporative demand in two experiments. We have finally tested to what extent the equations derived from dynamic analyses allow simulation of leaf length and width in 15 field experiments in Europe and 6 experiments in controlled environment. A genetic analysis performed in 251 maize hybrids in the field and in controlled environment is also presented to support the hypothesis of separate genetic controls for leaf length and width in response to light and evaporative demand.

Accepted Article

## **Material and methods**

### **Calculation of Intercepted radiation in the platform and in the field**

In all field experiments, the amount of light intercepted by canopies was simulated by using the crop model APSIM-maize (Hammer *et al.*, 2010) previously parameterised for the genotype B73 (Harrison *et al.*, 2014). Briefly, this model uses the Beer-Lambert approach, calculating intercepted light from the leaf area index and an extinction coefficient. We used for that daily meteorological data, sowing date and sowing density, maintaining optimal water and nitrogen conditions during simulations. The amount of light intercepted by individual plants was then calculated by using plant density.

In platform experiments, the amount of light intercepted by each plant was estimated as in Cabrera-Bosquet *et al.* (2016). Briefly, 3D plant architectures (point cloud with resolution = 0.512 cm<sup>3</sup>) were obtained from binarized RGB images with a space carving algorithm (Kutulakos & Seitz, 2000). These 3D plants were used to reconstruct the 3D maize canopy in the platform during the whole experiment. Intercepted light was calculated by using the RATP model (Sinoquet *et al.*, 2001).

### **Timing of leaf elongation and widening**

An experiment was performed in Mauguio (France, near Montpellier, SI Table S1) with the hybrid Déa at a density of 10 plants m<sup>-2</sup> irrigated twice a week. Incident radiation was measured every 15 minutes (LI-190SB, Li-Cor, Lincoln, USA), together with air humidity and temperature (HMP35A Vaisala Oy, Helsinki, Finlande; SI Table S1 and SI Table S3). Leaf appearance rate was measured in a set of 10 plants by scoring phenological stages every second day over the whole experiment. Destructive plant measurements were carried out on 10 plants every second day. Sampled plants were chosen to represent the median of phenological stage over the whole field. For each plant, width and length of each leaf were measured after plant dissection, thereby allowing measurement of leaves enclosed in the whorl. This was performed with a ruler when leaves were more than 4-mm long, and with image analysis connected to a binocular lens (Bioscan-Optimas V 4.10, Edmonds, WA) otherwise.

Daily mean values of leaf length and maximum width were used to estimate the increase in width and length over time. To compare chronological patterns of leaf width and length on the same scale, data were first normalized by the maximum width or length. A curve was then fitted on time courses with a local regression model ('loess' function in R, – span = 0.6). Thermal time was calculated as in the APSIM model (Hammer *et al.*, 2010).

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## Dynamic response of leaf elongation rate to environmental conditions

Three plants of the hybrid Déa were grown under well-watered conditions in a soil 40/60% (v/v) of clay and organic compost (SI. Table S3), in the greenhouse of the phenotyping platform *PhenoDyn* (Montpellier, France - <https://www6.montpellier.inra.fr/lepse/M3P> - SI. Table S2). The photoperiod was 14 hours day and 10 hours night with additional light used between 7h to 20h when light was below 450 W.m<sup>-2</sup>. Air temperature oscillated naturally during the day but was kept above 15°C at night and below 28°C during the day (mean value of 20.4 °C), and VPD reached 3.5 kPa in some afternoons (mean value of 0.54 kPa). Air humidity, air temperature (HMP35A Vaisala Oy, Helsinki, Finland) and leaf temperature (thermocouples) were measured every 15 minutes. Leaf elongation rate (LER) was measured every 15 min with rotating displacement transducers (RDTs 60-1045 Full Smart Position Sensor; Spectrol Electronics, Ltd, Wiltshire, England) as in Sadok *et al.* (2007). Plants were maintained at a soil water potential higher than -0.05 MPa via automatic irrigation. LER of leaf 6 was measured from leaf 6 appearance for 4 days (during stable maximum leaf elongation rate) with contrasted leaf-to-air vapour pressure deficit (VPD<sub>la</sub>) defined as the difference between saturation at leaf temperature (estimated as in Millet *et al.*, 2016) and actual vapour pressure in the air. LER was expressed per unit of thermal time at each 15 minute time step, with thermal time calculated as in the APSIM model (Hammer *et al.* 2010). The response of LER of leaf 6 to VPD<sub>la</sub> was estimated by using LER and VPD<sub>la</sub> data at the time step of 15 min for the 3 plants from 14h to 17h, i.e. during daily peaks of vapour pressure deficit. Because the relationship was tight (see results), this resulted in an equation relating LER to VPD<sub>la</sub>.

$$LER = a_i - VPD_{la} * b \quad (\text{Eq. 1})$$

With  $a_i$  (mm °Cd<sup>-1</sup>) the potential leaf elongation rate for the considered leaf rank  $i$  in well-watered conditions and low evaporative demand, and  $b$  (mm °Cd<sup>-1</sup> kPa<sup>-1</sup>) the sensitivity of LER to evaporative demand (leaf-to-air vapour pressure deficit VPD<sub>la</sub>), considered common for all leaf ranks. This equation was fitted on leaf 6 results, thereby allowing estimation of  $a_6$  and  $b$ . The potential LER for each leaf ( $a_i$ ) was inferred from  $a_6$  for each leaf rank  $i$  by using relationship of Chenu *et al.* (2008). At each time  $t$  between beginning and end of leaf elongation, the lamina length of leaf rank  $i$  ( $L_i$ ) was simulated as the cumulated leaf elongation rate at each time step:

$$L_i = \sum_0^t LER_i \quad (\text{Eq. 2})$$

The final leaf length was calculated at the end of leaf elongation ( $t = t_{\text{final}}$ ).

## Dynamic responses of leaf length and width to light

An experiment was performed in Grignon (France, near Paris, SI Table S1) with the hybrid Déa at a density of 10 plants m<sup>-2</sup>. The field (deep clay loam) was fully irrigated for the whole experiment. Minimum, maximum temperatures, incident light and VPD were recorded every day. Temperature varied naturally from 0.0°C to 25.2°C with mean value of 8.8°C and VPD reached 2.6 kPa (mean value, 0.5 kPa). Three plots of 10 plants were marked at emergence for identification of phenological stages (leaf tip and ligule appearances) every second day on ten plants per plot before the onset of experimental treatments.

One plot stayed under normal light conditions during the whole experiment (“Light”, daily mean value of 16.6 MJ m<sup>-2</sup>). The second plot was shaded from leaf-6 emergence to the end of the experiment with a horticultural net transmitting 20% of light for all wavelengths in the visible spectrum, placed at 30 cm above plant tops (“Shaded”). The same shading nets were used for the last treatment, alternating light and shade conditions (“Alternated”) by placing and removing the nets : shade from leaf 6 to leaf 10 emergence, normal light conditions from leaf 10 to leaf 14 emergence, and shade conditions afterwards. Final individual leaf area and shape were measured for all leaves of all plants at the end of stem growth by image analysis (Sinoquet, Moulia & Bonhomme, 1991).

A position-time model was established to relate each longitudinal position on the leaf to the time when the considered leaf element left the leaf growing zone because it was pushed by younger elements (Ben Haj Salah & Tardieu, 1995, Silk, 1992). The growing zone is located in the first 8 cm beyond the leaf insertion point in maize (Tardieu *et al.*, 2000). Leaf elongation rate was simulated by using Eq. 1, for which leaf length was predicted every hour from the beginning to the end of linear leaf elongation (Eq. 2). The beginning of leaf linear elongation was calculated as the time when the leaf reached 8 cm, estimated for each leaf as in Chenu *et al.* (2008). A relationship could therefore be established, for each time step, between leaf length and time after beginning of leaf linear elongation. Each longitudinal position along the leaf could therefore be attributed a time after the corresponding leaf element left the leaf growing zone (see Ben Haj Salah & Tardieu, 1995). We could therefore predict at which time the width at any leaf position was produced and identify the environmental conditions at that time, and finally relate these conditions to the width at the corresponding position. This has been performed for leaves 6 to 9 in the three treatments.

At each time after beginning of linear leaf elongation, a ratio was defined for comparing leaf widths produced in the alternated treatment to those in the shade and light treatments.

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$$\text{Width ratio} = (W_{\text{alternated}} - W_{\text{shade}}) / (W_{\text{light}} - W_{\text{shade}}) \quad (\text{Eq.3})$$

This ratio is equal to 0 when leaf width of plants in the alternated treatment matches that of shaded plants and 1 when it matches that of plants under full-light.

Because we observed a dynamic relationship between leaf widening and whole-plant intercepted light during the period of widening, this resulted in the equation:

$$W = W_{\text{min}, i} + k R_{\text{int}} \quad (\text{Eq. 4})$$

With  $W_{\text{min}, i}$  the x-intercept (minimum leaf width under low light) of the considered leaf  $i$ , and  $k$  the sensitivity to intercepted radiation, considered common for all leaves.

### External dataset in the platform and the field

Fifteen field experiments were carried out in a West-East transect across Europe and one site in Chile in 2011 to 2013 (DROPS network – Millet *et al.*, 2016, SI.Table S1, SI.Table S3) with the maize hybrid B73 x UH007 (named 'B73' afterwards). Soil water status was measured every second day and maintained higher than -0.1 MPa by irrigation (SI. Table S3). Nitrogen and pesticides application were performed following local practices. Air temperature and humidity, wind speed and incident light were recorded every hour in each experiment. Raw measurements were first converted to common units and compared to local meteorological data to assess the dataset (Millet *et al.* 2016). Vapour pressure deficit was calculated hourly using temperature and humidity, corrected by radiation in low light conditions as in Chenu *et al.* (2008). Environmental conditions are summarised for each experiment in SI.Table S3. The network of field experiments showed a large variety of scenarios of temperature, vapour pressure deficit and incident light through the growing season as presented in Millet *et al.* (2016) (detail in SI Table. S3). In each of the 15 experiments, the final length (from ligule to leaf tip) and final width (maximum over the leaf) of leaves 8 to 11 were measured in 10 plants per experiment.

Six experiments were carried out in the phenotyping platform *PhenoArch* (Montpellier, France, Cabrera-Bosquet *et al.*, 2016; <https://www6.montpellier.inra.fr/lepse/M3P>) from 2011 to 2016, during different seasons of the year (SI. Table S2). Three seeds per pot of the hybrid B73 were sown at 0.025m depth, and thinned to one plant per pot at three-leaf stage. Plants were grown in 9L PVC pots in a substrate composed of a mixture of clay and organic compost (30/70 volume). Soil water content was maintained at retention capacity in each pot by daily watering (soil water potential of -0.05MPa). Briefly, the *PhenoArch* platform is equipped with automated weighting and watering stations, imaging stations and environmental sensors. For each plant, 13 RGB images (2056×2454

pixels) were taken every night (one from top and 12 side images with a 30° horizontal rotation). Temperature and  $VPD_{ia}$  were recorded every 15 min in 8 sites of the greenhouse (SI. Table S3). Incident light was estimated at each individual plant position as in Cabrera-Bosquet *et al.* (2016) from incident light outside the greenhouse and local extinction coefficients. Daily mean temperature in the greenhouse was  $18 \pm 2$  °C (night) and  $25 \pm 3$ °C (day), with small differences between experiments. Incident light varied between experiments, with maximum values ( $7.7 \text{ MJ.m}^{-2}.\text{day}^{-1}$ ) was measured in *Spring 2016* and lowest in *Autumn 2011* ( $2.8 \text{ MJ.m}^{-2} \text{ day}^{-1}$ ).

The B73 hybrid was grown in all experiments (from 2 to 3 replicates, SI. Table S2), together with other 251 hybrids (Millet *et al.* 2016) (SI. Table S2). In each experiment, leaf width was measured on hybrid B73 by image analysis on ligulated leaves from rank 7 to 12 for 2 to 5 plants per experiment (SI. Method S1). It was compared to planimeter measurements (LI-3100C Area Meter) in plants of the experiment Spr16 ( $R^2 = 0.93$ , not shown).

The dynamic relationships determined previously (Eq.1 and Eq.4) were tested in this external dataset. We have first tested over the whole dataset the relationships between final dimensions and environmental variables, considering a unique sensitivity to environmental conditions for all leaves.

Environmental variables were calculated as the conditions sensed by plants during the growth of each individual leaf. The effect of leaf rank was calculated as the intercept of the relationship for each leaf. The test of the model itself consisted in simulating final leaf dimensions with the equations determined in dynamic experiments (Eq. 1 and Eq. 4) and comparing with observed values.

### Genetic analyses

Experiments were performed in the field and in the platform with a panel of 251 maize hybrids (DROPS panel – Millet *et al.* 2016) obtained by crossing 251 dent lines with a common flint parent (UH007). The panel structure is further described in Millet *et al.* (2016). In the field (Saint-Martin de Hinx (France, SI. Table S1), plants were sown on 12<sup>th</sup> May at a density of  $8.5 \text{ plants m}^{-2}$  with 10 plants of each genotype sown in a row and soil water status maintained at high values by irrigation. Environmental conditions and crop management were collected as above and are summarised in SI. Table S3. The leaf length and width of leaves 7 to 10 were measured within 3 days before flowering for three plants per hybrid. Leaf width and length were also measured in the platform experiment on the 251 genotypes (Exp. Spr16) for leaves 7 and 8.

Genotypic means were calculated for each hybrid using a mixed model based on spatial design:

$$y = Z_g \tau_g + Z_c u_c + Z_r u_r + e \quad (\text{Eq. 5})$$

where  $y$  is the vector of phenotypic observations in a given experiment,  $\tau_g$  is the vector of fixed genotypic effect, and  $u_c$  and  $u_r$  are vectors of random column and row effects, which are used to capture linear trends that might exist across rows and columns. The residual error  $e$  was assumed to follow a zero mean multivariate normal distribution, with covariance determined by autoregressive processes of order one in the row and column direction. Calculations were performed with ASReml-R (Butler *et al.*, 2009). Narrow-sense heritability was estimated with a model assuming additive SNP effects using the R-package *Heritability* (Kruijer *et al.*, 2015).

Lines were genotyped using a 50K Infinium HD Illumina array (Ganal *et al.*, 2011), and a 600K Axiom Affymetrix array (Unterseer *et al.*, 2014). The genetic analysis was performed with the single-environment method (GWAS, Genome Wide Association Study) presented in Millet *et al.* (2016) with FaST-LMM v2.07 (Lippert *et al.*, 2011) on individual traits for each experiment using the single locus mixed model:

$$Y = \mu + X\beta + G + E \quad (\text{Eq. 6})$$

where  $Y$  is the vector of phenotypic values,  $\mu$  the overall mean,  $X$  is the vector of SNP scores,  $\beta$  is the additive effect, and  $G$  and  $E$  represent random polygenic and residual effects. The variance-covariance matrix of  $G$  was determined by a genetic relatedness (or kinship) matrix, derived from all SNPs except those on the chromosome containing the SNP being tested as described in Millet *et al.* (2016) following the method of Rincent *et al.* (2014). The SNP effects  $\beta$  were estimated by generalized least squares, and their significance ( $H_0: \beta = 0$ ) tested with an F-statistic. Candidate SNPs distant less than 0.1 cM were considered as belonging to a common QTL, described via the most significant SNP in the QTL and the interval between all SNPs belonging to the QTL. Co-localisations between QTLs were checked by comparing QTLs intervals and checking for overlap (defined by all SNPs contained in the QTL). Physical positions of significant SNPs were projected on the consensus genetic map for Dent genetic material (Giraud *et al.*, 2014).

## Results

### *Different time courses for leaf elongation and widening.*

The time courses of elongation and widening were analysed in a field experiment in which leaves 8 to 11 of ten plants were sampled every second day from leaf initiation to end of elongation, after dissection of the plant whorl. The increase in leaf length, defined as the distance from the leaf insertion point to the tip, was delayed compared to the increase in leaf width (Fig. 1). The beginning of rapid elongation (5% of final leaf length) occurred 35°Cd later than the beginning of rapid leaf widening (5% of final leaf width). Cessation of elongation (95% of final length) occurred 39°Cd after cessation of widening. This temporal pattern, presented in Fig. 1 for leaf 8, was also observed in leaves 6, 7, 9, 10 and 11 (SI Fig.S1). In analyses presented below, the effects of environmental conditions were therefore tested during the respective periods corresponding to 5-95% of either elongation or widening.

### *Leaf width increased with intercepted light with no effect of evaporative demand*

Shading largely affected leaf width in a field experiment where plants were grown either under natural light conditions ("Light"), shaded (80% light attenuation from the appearance of leaf-6 tip onwards, "Shaded") or undergoing alternating periods of normal light and shade ("Alternated"), with shading from leaf-6 appearance to leaf-10 appearance and from leaf-14 appearance onwards (Fig.2, 3). Plants with maximum incident light had wider leaves than those permanently shaded (Fig.2c; SI.Fig.2). Shading during part of the period of leaf widening (Fig.3a) resulted in intermediate values (Fig.2c) and suggested a dynamic effect because differences between treatments changed with time (Fig. 2a). This effect was analysed via a position-time model that allowed associating each longitudinal position on the leaf to a time after beginning of linear elongation. New cells are continuously produced in the 3-cm-long meristem close to the leaf insertion point and flow through the 8-cm-long growing zone to mature zones (Tardieu *et al.*, 2000). One can therefore assign, to each longitudinal position on the leaf (Fig.2a), a time after the considered cells have left the elongation zone (Eq. 1 and 2, SI.Fig.3). A width ratio (Eq.3) was defined for comparing leaf widths in the three treatments at any longitudinal position of the leaf. It equalled 100% if width were equal in 'Alternated' and 'Light' treatments and 0% if width were equal in 'Alternated' and 'Shaded' treatments (Fig. 3a). This was performed for leaves 7 to 9 whose development phases were shifted by 35 °Cd per leaf rank.

The width ratio of leaf 7 rapidly decreased when plants were shaded and increased after full light was restored (Fig. 3a). Because the beginning of leaf expansion differed for each leaf rank, leaves of

different ranks underwent different timing of full-light and shading in the 'Alternated' treatment. The width of leaf 8 decreased when plants were shaded and increased under full light after a lag time. Leaf 9 started growth just before full-light, with rapid increase of the width ratio, followed by a stabilization of this ratio at the beginning of the second period of shading. Overall, the final leaf width corrected for the effect of leaf rank was closely related to the cumulated light intercepted per plant for the period with maximum widening rate (Fig 3b; calculated with the crop model APSIM-maize). It is noteworthy that a common calculation was adopted for intercepted light per plant in the three treatments without considering the effect of light on leaf width, to avoid circular reasoning (i.e. effect on light interception on leaf width and effect of leaf width on light interception).

The validity of the relationship between intercepted light per plant and leaf width (Fig. 3b; Eq.4) was tested in an external dataset of 15 experiments in the field and six experiments in the phenotyping platform. A large range of environmental conditions was observed (SI. Table 3), with lower values of intercepted light per plant and evaporative demand in the greenhouse than in the field. Leaf width was significantly higher in the field than in the greenhouse for leaves 8 to 11 (50% of mean values for each leaf rank, SI. Fig.4). For a given leaf rank, it was closely related to the amount of light intercepted by plants for the period elapsing from 5% to 95% final width. A common relationship between leaf width and intercepted light per plant accounted for variations in width between field experiments and for the difference between field and greenhouse (Fig. 4a). Leaf width was also negatively related to minimum and mean temperatures (Table 1), probably via the effect of temperature on the amount of light intercepted over the considered period of development. Indeed, because the latter has a constant duration in thermal time, its duration in calendar time decreased with temperature. Leaf width showed no relationship with vapour pressure deficit (Table 1).

#### *Leaf length decreased with evaporative demand and light*

Leaf elongation was analysed in the shading experiment presented above and in one experiment in controlled environment. In the latter, the elongation rate of leaf 6 was maximum during the night, with no light and low  $VPD_{ia}$ , and minimum during periods with high light and  $VPD_{ia}$ . Plants reacted within minutes after changes in environmental conditions with a strong negative effect of  $VPD_{ia}$  on leaf elongation rate (Fig. 5a,b). Leaf elongation rate measured every 15 min was closely related with  $VPD_{ia}$  over the same periods (Fig. 5c), probably via a negative hydraulic effect decreasing leaf water potential in the leaf growing zone (Tardieu, Simonneau & Parent, 2015).

Shading had opposite effects on leaf length and width in the shading experiment (Fig.2b,c, Fig.S2). Final leaf length of plants growing under shading was longer by 6 to 14 cm compared to those grown

under full light, with intermediate values for alternated shading (Fig. 2b). In the platform experiment, light had a negative effect on leaf elongation rate, probably via its contribution to  $VPD_{ia}$  due to leaf heating and, eventually, its effect on leaf water potential (Ben Haj Salah & Tardieu, 1996).

We have tested if the relationship between leaf elongation rate (LER) and  $VPD_{ia}$  in the greenhouse (Eq. 1) may account for variations of final leaf length in 15 experiments in the field and one experiment in the platform. Final leaf length was closely related to the mean value of afternoon  $VPD_{ia}$  over the period of maximum leaf elongation. The latter was calculated in each field based on the thermal times at which leaf length reached 5 and 95% of their final values according to Fig. 1. A clear relationship was observed between fields, which also accounted for the difference between field and greenhouse (Table 1 and Fig. 4b). Final leaf length had no relationship with intercepted light per plant or with mean temperature over the same period.

*Taking into account environmental effects on leaf length and width largely improved the prediction of individual leaf area.*

The results presented above resulted in Eq. 1 to 4, with parameters established in short term experiments. These equations adequately predicted leaf length and width observed in the independent dataset of field and platform experiments carried out over the whole vegetative period (Fig. 4) with CV of 7% and 11% for leaf length and width, respectively. The model of leaf width based on intercepted light captured a large part of the observed variance, with predictions highly improved ( $R^2=0.71$ , CV of error = 11%) compared to the algorithm in which leaf width would be considered as proportional to leaf length ( $R^2 = 0.01$ , CV of errors = 26%). This resulted in an adequate prediction of individual leaf area in the whole field dataset (Fig. 6c,  $R^2=0.62$ , CV = 16%), compared to the model in which leaf width was calculated as a proportion of leaf length ( $R^2=0.04$ , CV = 34% ).

*The genetic controls of leaf length and width were largely independent, with consistent allelic effects in field and platform experiments.*

Genetic analyses were performed on the mean length and width of leaves 7 and 8 in the platform and of leaves 8 to 10 in the field, in a panel of 251 maize hybrids. Heritabilities were medium to high, except for width measured in the field (Table 2b). The width of leaf 8 largely varied from 5.1 to 9.9 cm in the field and from 5.6 to 8.3 cm in the platform. Correlations between leaf width and leaf length were low in both platform and field experiments ( $R^2 = 0.001$  and 0.26, Table 2). Measured leaves were longer and narrower in the platform than in the field (respectively +30% and -26% for leaf 8; mean value for all genotypes), consistent with the difference in intercepted light per plant

and evaporative demand (Fig. 7). Hence, the effects studied in detail in one hybrid applied to the whole panel.

GWAS analysis led to the identification of twenty nine and fourteen significant QTLs in our dataset for leaf width and leaf length, respectively, either in the field or in the platform (Table 3).

We have first tested if QTLs that affected leaf length or leaf width in the field also affected the same trait in the platform and vice versa. For that, we have considered allelic effects of all QTLs in field and platform, regardless of their significance in the considered condition (by allelic effect, we mean the effect of the allele of the reference hybrid B73 vs the other allele at each QTL, expressed as a percentage of the general mean). This can be visualized in Table 3 (upper part) in which the allelic effects of the 15 QTLs of leaf width identified in the field are also presented for leaf width in the platform and the 15 QTLs identified for leaf width in the platform are also presented for the field. A visual inspection shows that QTLs that positively affected leaf width in one environment also positively affected it in the other one (consistent colours in Table 3). Allelic effects had contrasting amplitudes in the field and in the platform, so most significant QTLs in the field did not reach the threshold of significance (only one colocation of significant QTLs). However, allelic effects were clearly correlated between experiments, for instance allelic effects of QTLs of width identified in the platform were highly correlated with allelic effects of the same QTLs in the field ( $R^2=0.88$ , and  $R^2=0.68$  for the reciprocal analysis). The same conclusion applied to QTLs of leaf length, in which the nine QTLs of leaf length identified in the field also affected leaf length in the platform, with consistent signs of allelic effects and a high correlation ( $R^2=0.91$ , and  $R^2=0.91$  for the reciprocal analysis). Hence, we can conclude in a largely common genetic control in the field and in the platform for both leaf width and leaf length, in spite of a high QTL x experiment interaction.

We have then tested if QTLs of leaf width also affected leaf length and vice versa. Overall, this was not the case because QTLs which had high allelic effects on leaf width had small effects on leaf length and vice versa ( $R^2=0.18$ ). This can be visualized in Table 3 via the difference in colour and colour intensity between the left and right parts of the table. Hence, we can conclude that the genetic controls of leaf width and leaf length were largely independent.

## Discussion

This study reconciles the results on the effects of light interception on leaf growth in dicotyledonous and monocotyledonous species. Whereas a clear effect was shown in dicotyledonous species (Granier & Tardieu, 1999a,b, Pantin, Simonneau & Muller, 2012, Pantin *et al.*, 2011), most physiological studies on monocotyledons concluded in a strong effect of hydraulics and an absence of effect of intercepted light (Ben Haj Salah & Tardieu, 1997, Mahdid *et al.*, 2011, Parent *et al.*, 2010). This was because leaf width was not taken into account in these studies. Here, both short-term dynamic experiments and statistical analyses of a large dataset in the field and in phenotyping platform conclude in a clear effect of light interception on the growth of individual leaf area.

Leaf width and length are under different genetic and physiological controls. The lack of genetic correlation between length and width is consistent with studies on biparental populations (Wei *et al.*, 2016, Yang C. *et al.*, 2016), but this study extends them to the dent maize populations originating from breeding programmes in the USA and Europe and, potentially, to the whole maize species. We also confirm the large variability of leaf width from both genetic and environmental points of view. Within the few studies considering leaf width, most of them have shown that a larger part of variance of individual leaf area in biparental population was explained by leaf width compared to that by leaf length (Wei *et al.*, 2016, Yang C. *et al.*, 2016). This contrasts to a study in wheat (Yang DL. *et al.*, 2016), working with biparental population found that leaf width was less variable compared to leaf length. In our diversity panel, we found an even larger importance of leaf width on individual leaf area compared to that of leaf length (85% vs 15 % of explained variance). Several underlying mechanisms could explain the differences in responses of leaf elongation and widening. It has been shown that lower water status results in shorter cells in maize (Ben Haj Salah *et al.*, 1997) but at the present time and in our knowledge, there is no evidence in favour of the hypothesis of larger cells of more cell rows due to higher intercepted radiation.

The impact of variation of leaf width in multi-environment field trials was also larger than expected. It contrasted with the results of Chenu *et al.* (2008) that did not take into account the variations of leaf width for predicting plant leaf area in Australian maize experiments, but still obtained an adequate prediction of leaf area. We can hypothesise that fields in Australia received high and relatively similar light, resulting in similar leaf widths. Conversely, the experiments presented here, which covered a large part of the European maize growing area presented large differences in light, resulting in large impacts on leaf width and area. Overall, this study to some extent reconciles the results of short-term experiments, which have focused on leaf length with essentially hydraulic effects, with those of a family of crop models that involve carbon supply in the control of leaf area.

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Indeed, short-term experiments found an effect of intercepted light per plant on leaf area, provided that leaf width is taken into account.

Extrapolating relationships observed at different levels of integration (temporal, spatial) was successful here although they are largely debated (Passioura, 1996, 2007, Poorter *et al.*, 2016). Indeed, many processes show trades-off between rates and duration. For instance, temperature has a large effect on leaf elongation rate of several species, but a very low effect on final length because of a total compensation of rate by duration (Parent & Tardieu, 2012). The success in the upscaling performed here probably involves that such compensations had a minor role in the effects of environmental conditions on leaf width and length. This opens the way to a dialogue between platform and field experiments for the prediction of the genetic variability of the control of leaf growth, in spite of the fact that traits largely differ between field and controlled environment (Poorter *et al.*, 2016).

A crucial question in crop modelling is whether adding complexity to the system could significantly improve simulations (Parent *et al.*, 2016). It is therefore a relevant modelling question if both leaf widening and elongation have to be considered compared to simpler formalisms. At present, in most crop models, two categories of algorithms have been proposed, giving contrasting roles for water vs carbon availabilities in the control of leaf growth. The first category considers that the sensitivity of leaf expansion to environmental conditions is indirect and acts through photosynthesis and carbon allocation (e.g. GECROS; Yin & van Laar, 2005). The second category considers direct empirical responses to environmental conditions, regardless of carbon availability, either at the whole-plant level (big leaf models, e.g. APSIM-maize, Hammer *et al.*, 2010) or at individual leaf level. (e.g. Chenu *et al.*, 2008, Lawless, Semenov & Jamieson, 2005, Lizaso *et al.*, 2003, 2011). All these models consider the expansion of individual leaf area as a whole, thereby implicitly considering that, in monocotyledons, differential responses of leaf widening and elongation to environmental variables do not need to be distinguished. The same implicit assumption can be found in (Chenu *et al.*, 2008, 2009) that simulated the impact of QTLs of maize leaf elongation on yield, without considering that leaf width may respond differentially to environment. By contrast, this study shows that it can be a relevant choice to consider separately leaf widening and elongation in the range of studied situations and genotypes, which caused large variations of leaf width. Because the quality of simulations of crop models are linked to the goodness of leaf area predictions (Martre *et al.*, 2015), we propose that identifying leaf widening and elongation in a plant model and therefore improving leaf area predictions could significantly improve predictions. This conclusion is consistent with physiological considerations, namely that the controls of carbon-driven and hydraulic- driven

mechanisms at the cellular scale, are under the control of very different genes, and translating into contrasted sensitivities of final leaf length and width to environmental conditions in different genotypes.

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### **Author Contributions**

S.L. analyzed most data

C.F. performed dynamic experiments on width

C.P supervised the field experiments for genetic analyses

E.M. analyzed data on mapping population

B.P. and F.T. planned and designed the research

B.P., S.L. and F.T. wrote the manuscript.

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**Table 1:** Correlations of environmental variables with leaf width and length.

	$T_{max}$		$T_{min}$		$T_{mean}$		$VPD_{max}$		$VPD_{min}$		$VPD_{mean}$		$R_n$	
	r	pvalue	r	pvalue	r	pvalue	r	pvalue	r	pvalue	r	pvalue	r	pvalue
Width	0.29	0.01	-0.71	<0.001	-0.66	<0.001	0.04	0.73	-0.09	0.4	-0.02	0.83	0.82	<0.001
Length	-0.04	0.19	-0.15	0.09	0.19	0.72	-0.4	<0.001	-0.04	0.23	-0.29	<0.01	-0.09	0.67

The coefficient of correlation (r) was calculated with the R function “corr”, and corresponding p-values were calculated with the “cor.test” function. Data is summarized from leaf beginning to end of expansion: mean of daily minimum temperature, mean of maximum temperature and mean temperature ( $T_{min}$ ,  $T_{max}$  and  $T_{mean}$ ); mean of daily minimum, mean of maximum and mean leaf-to-air vapour pressure deficit ( $VPD_{max}$ ,  $VPD_{min}$  and  $VPD_{mean}$ ); mean of daily incident light ( $R_n$ ). Significant threshold was fixed at a p-value of 0.01 (p-value > 0.01 : non-significant ; p-value  $\leq$  0.01 : significant ; p-value  $\leq$  0.001 : highly significant ).

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**Table 2:** Coefficient of correlation between leaf length and width (a), narrow sense heritability, number of QTLs identified by the GWAS analysis and prediction capacity of each set of QTLs on the measured variable (b).

(a)		Platform		Field	
		Length	Width	Length	Width
Platform	Length	1	0.03	0.24	-0.14
	Width		1	0.06	0.42
Field	Length			1	0.51
	Width				1

(b)		Trait	Heritability	QTLs	Prediction
Platform	Length		0.80	5	0.35
	Width		0.73	16	0.64
Field	Length		0.48	10	0.19
	Width		0.10	15	0.39

Data come from 251 genotypes for one platform experiment and one field experiment. (b) For each set of QTLs on the measured variable in the field or in the platform.

**Table 3:** Comparison of allelic effects of QTLs identified for leaf width or length in the platform and in the field.

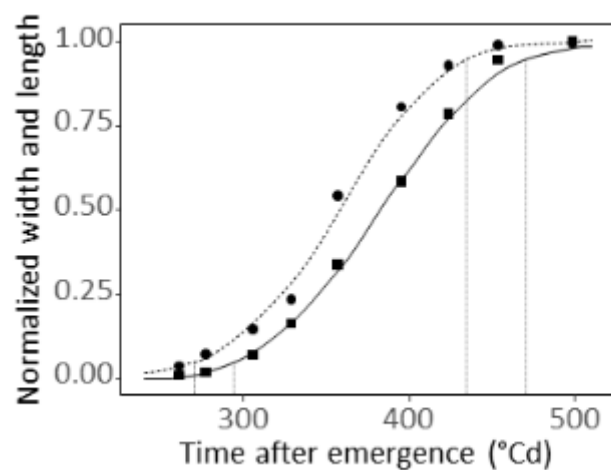
Variable	SNP name	Bin	Position (cM)	Effect on width (%)		Effect on length (%)	
				Field $R_{int} = 1.4$ MJ/m <sup>2</sup>	Platform $R_{int} = 0.4$ MJ/m <sup>2</sup>	Field VPD = 2.4 kPa	Platform VPD = 0.8 kPa
Field Width	S1_14810807	1.02	35.2	2.2*	0.1	0.7	-0.5
	AX-90656316	1.02	53.4	2.4*	0.4	0.3	-0.3
	S1_204867908	1.07	155.9	-2.3*	-1.6	-1.3	0.0
	S1_205242298	1.07	156.2	2.5*	1.9	0.9	0.2
	AX-91404589	2.06	128.3	2.2*	1.3	0.6	0.1
	AX-91846273	2.06	129.5	2.4*	1.5	0.8	0.3
	AX-90618756	2.07	159.5	2.4*	1.0	0.0	0.1
	AX-90782812	2.07	160.1	2.3*	1.2	0.0	-0.2
	AX-90841086	3.06	111.4	2.3*	1.0	1.2	0.5
	AX-90841149	3.06	111.6	2.3*	1.0	1.2	0.5
	AX-91623171	4.05	55.7	2.4*	1.5	0.0	-0.4
	AX-90977036	5.08	187.1	-2.3*	-0.5	0.0	1.1
	SYN19549	10.1	87.9	2.3*	1.0	0.6	0.0
	S10_141613269	10.1	109.6	-2.3*	-1.0	-1.2	-0.4
AX-91194831	10.1	116.8	2.3*	-0.2	0.0	-0.5	
Platform Width	AX-90700654	1.06	147.8	-1.4	-2.8*	0.0	-0.9
	AX-90700772	1.06	148.3	-1.0	-2.5*	0.3	-1.2
	AX-91402250	1.07	156.5	-2.1*	-2.7*	0.1	0.1
	AX-90610562	1.08	189.7	-1.5	-3.1*	-0.9	-0.5
	AX-90733206	2.02	39.1	-1.1	-2.7*	0.2	0.3
	S2_11564804	2.02	39.7	1.4	2.6*	-0.3	-0.6
	AX-91388874	2.02	42.8	-1.4	-2.6*	0.0	0.3
	AX-90598044	2.03	59.1	0.7	2.7*	0.3	-0.1
	AX-90775864	2.06	110.6	0.6	2.9*	-0.2	-0.1
	AX-91541880	2.06	111.0	-0.7	-2.7*	-0.3	-0.2
	PZE-102124387	2.06	111.2	-0.8	-2.7*	-0.4	-0.1
	AX-90776150	2.06	111.9	-1.1	-2.7*	0.1	-0.1
	AX-91542155	2.06	112.8	-0.7	-2.7*	-0.3	-0.2
	AX-91412182	6.01	12.5	0.8	2.8*	0.4	0.1
S8_172254661	8.08	170.6	0.9	2.7*	-0.2	0.0	
Field Length	AX-91418229	1.08	200.5	1.9	0.8	1.8*	0.7
	AX-90716443	1.09	207.6	-1.2	-0.3	-1.8*	-1.0
	AX-90602493	4.03	41.2	-1.1	-0.6	-1.9*	-0.9
	AX-90896424	4.06	63.2	-0.4	0.5	-2.0*	-0.8
	AX-90547782	5.07	149.7	0.9	0.9	2.1*	1.0
	S6_103026220	6.03	42.2	1.1	0.4	1.9*	0.5
	S6_113779929	6.04	56.1	-0.6	-1.2	-2.0*	-1.3
	PZE-108093081	8.06	129.4	0.5	-0.5	1.8*	1.0
AX-90624676	8.06	145.4	-0.7	-1.2	-1.9*	-0.4	
Platform Length	AX-91488109	1.06	141.9	-0.7	0.7	-0.1	2.0*
	AX-91525911	2.05	95.0	1.5	2.9	-0.2*	0.5*
	AX-90575329	5.05	108.3	-0.1	-0.5	-0.9	-2.0*
	S9_90366933	9.03	59.2	-0.1	1.1	0.2	2.0*
	AX-91445646	9.05	81.6	0.3	-0.6	-0.9	-1.8*

Data come from leaf width and length measurements on 251 genotypes for one platform experiment and one field experiment. The first column ("Variable") indicates the trait and

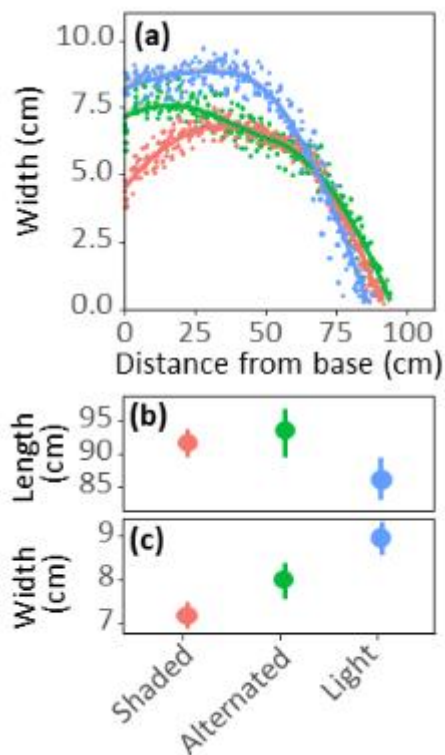


experiment for which the QTL has been detected significantly, together with its associated LOD score. The allelic effects of the same QTLs are presented for length and width in field and platform, regardless of the significance of the QTL in the considered trait and environment. Red and blue, positive and negative allelic effects (respectively) of the allele carried by genotype B73. \* indicates significant effect (LOD >5).  $R_{int}$  is the cumulated intercepted radiation during leaf widening and VPD is the mean value of leaf to air vapour pressure deficit during the period of leaf elongation.

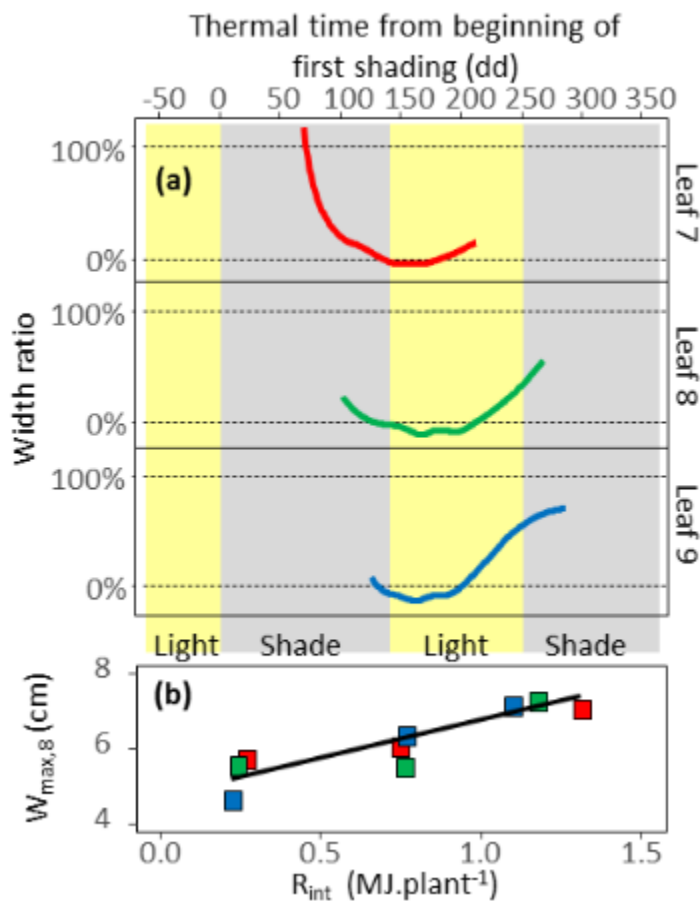
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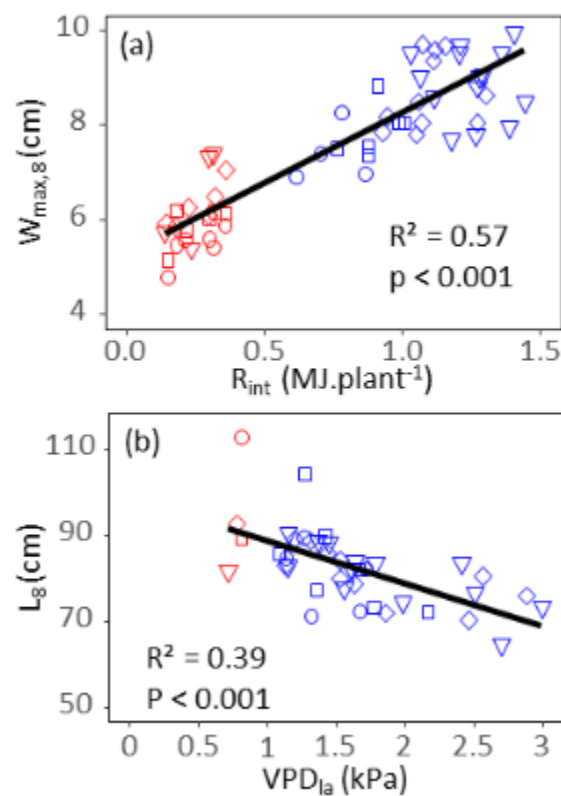
**Figure 1** : Time courses of widening and elongation of leaf 8. Circle and square dots: mean of leaf width and length for 10 plants per date (data are normalized by maximum value). *Dashed and solid lines* are local regressions (“loess” function) for width and length, respectively. Grey vertical dotted lines show 5% and 95% thermal time thresholds for each variable.



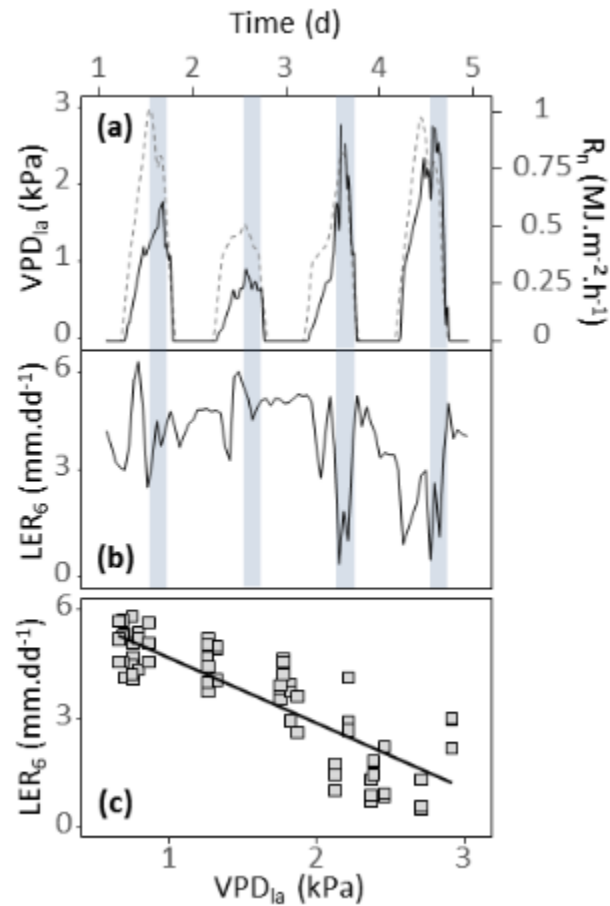
**Figure 2** : Leaf-8 shape in three light treatments. Leaf width plotted against the distance from leaf ligule (a), mean values for maximum leaf length (b) and width (c) for 10 plants. Blue : control treatment without shade; Red: plants continuously shaded since leaf-6 appearance; Green: alternated treatment between shade and light. In (a) Points: experimental data. Lines: local regression (fonction “loess”, span = 0.3). (b, c) Mean values and standard deviation of maximum leaf length and width.



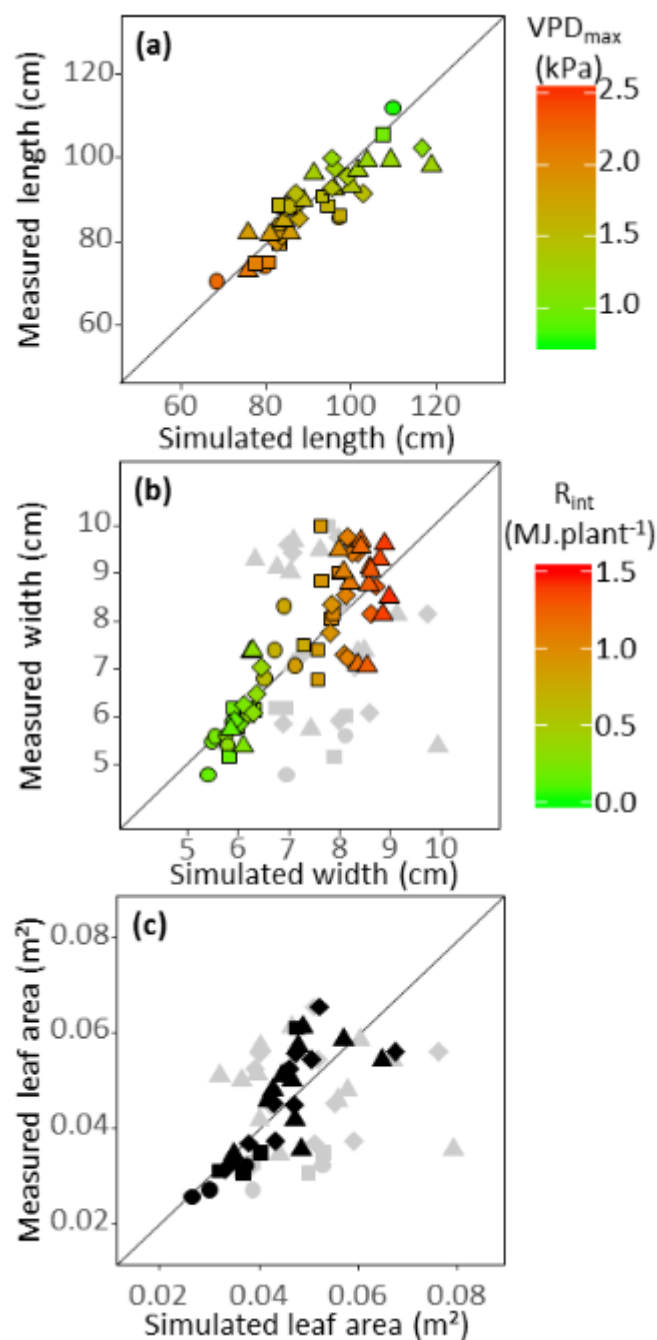
**Figure 3** : Response of leaf widening to intercepted light. (a) Leaf widening in the alternated treatment, relative to the full-light and shaded treatments in leaves 7 to 9. On the y-axis, width values in the alternated treatment are normalized by the difference between control and shade treatments, and showed as a percentage. 0% stands for leaf width in the shaded treatment (80% light reduction) and 100% for the full-light treatment. See text for position-time correspondence (b) Maximum leaf width plotted against plant intercepted light ( $R_{int}$ ) in the three treatments and three leaf ranks. ). Leaf width are corrected for leaf rank; ( $W_{max,8}$ ; see M&M). Colours displays the different leaf numbers as in (a).



**Figure 4** : Response of (a) leaf width to light intercepted by plants during leaf widening ( $R_{int}$ ) and (b) leaf length to leaf to air vapor pressure deficit during leaf elongation ( $VPD_{la}$ , mean of maximum daily values). Blue dots : Field data. Red dots: Platform data. Symbols represent leaf ranks from 8 to 11, circles, squares, diamonds and triangles, respectively. Leaf width and length values of the four leaf ranks are corrected for leaf rank so equivalent values for leaf 8 are presented (see M&M). Black lines, linear regressions.



**Figure 5:** Time courses of vapour pressure deficit (VPD<sub>la</sub>) (a, solid black line), light intensity (R<sub>n</sub>) (a - dashed grey line) and leaf elongation rate (b) during a 4-d experiment in the platform *PhenoDyn* in well-watered conditions. Data are smoothed with local regression (function loess, span = 0.02). Grey rectangles periods from 14 and 17h, during which VPD<sub>la</sub> has been taken into account (c) Response of leaf elongation rate (raw data, non-smoothed) to VPD<sub>la</sub> during highlighted periods.



**Figure 6** : Observed vs simulated final leaf length, width and area. Circles : leaf 8 ; Squares : leaf 9 ; Diamonds : leaf 10 ; Triangles : leaf 11.

**a)** Leaf length. RMSE = 6.21 cm, CV = 7%. Colours display  $VPD_{la}$  during leaf elongation.

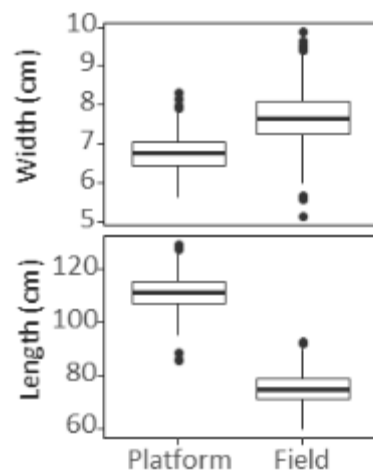
**b)** Leaf width. Grey dots represent leaf width if calculated as a fixed proportion of leaf length (RMSE = 1.95 cm, CV = 26%). Other colors, leaf width calculated from intercepted light per plant during leaf widening. RMSE = 0.8 cm, CV = 11%.

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c) Leaf area. Grey dots as in b, RMSE = 0.02 m<sup>2</sup>, CV = 34%. Black dots, leaf width calculated from intercepted light per plant during leaf widening. RMSE= 0.007m<sup>2</sup>, CV = 16%.

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**Figure 7** : Boxplot of measured maximum leaf width and final leaf length for leaf 8 for one platform experiment and one field experiment, for 251 maize hybrids.