Short title

Drought affects organ growth but not coordination

Title

Drought affects the rate and duration of organ growth but not inter organ growth coordination

Lennart Verbraeken^{a,b}, Nathalie Wuyts^{a,b,1}, Stien Mertens^{a,b}, Bernard Cannoot^{a,b}, Katrien Maleux^{a,b,2}, Kirin Demuynck^{a,b}, Jolien De Block^{a,b}, Julie Merchie^{a,b}, Stijn Dhondt^{a,b}, Gustavo Bonaventure^c, Steven Crafts-Brandner^{d,3}, Jonathan Vogel^d, Wesley Bruce^d, Dirk Inzé^{a,b}, Steven Maere^{a,b,*} and Hilde Nelissen^{a,b,*}

^a Ghent University, Department of Plant Biotechnology and Bioinformatics, 9052 Ghent, Belgium

^b VIB Center for Plant Systems Biology, 9052 Ghent, Belgium

^c BASF SE, Ghent, Belgium

^d BASF Corporation, 26 Davis Drive, Research Triangle Park, NC 27709, USA

* Senior authors: Steven Maere and Hilde Nelissen

Corresponding author

Hilde Nelissen

VIB Center for Plant Systems Biology

Ghent University, Department of Plant Biotechnology

Technologiepark 71

B-9052 Ghent (Belgium)

Tel.: +32 9 3313523

ORCID IDs: 0000-0001-8028-7088 (L.V.); 0000-0002-4445-507X (N.W.); 0000-0001-9192-1806 (S.M.); 0000-0001-9982-0890 (B.C.); 0000-0002-6564-9981 (K.M.); 0000-0002-8772-3505 (K.D.); 0000-0003-0047-4380 (J.D.B.); 0000-0003-1735-5118 (J.M.); 0000-0003-4402-2191 (S.D.); 0000-250001-6120 3897 (G.B.); 0000-0001-8550-3923 (J.V.); 0000-0001-8743-0377 (W.B.); 0000-0002-263217-8407 (D.I.); 0000-0002-5341-136X (S.M.); 0000-0001-7494-1290 (H.N.)

One-sentence summary

When affected by drought, the plant responds by adjusting the growth of individual organs but not the coordination between the organs.

Footnotes:

¹ Present address: IBG-2: Plant Sciences, Forschungszentrum Jülich GmbH, 52425 Jülich, Germany

² Present address: Aphea.Bio, 9052 Ghent, Belgium

³ Present address: 303 Oxpens Road, Cary, NC 27513

The authors responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiol.org) are: Hilde Nelissen (hilde.nelissen@psb.vib-ugent.be) and Steven Maere (steven.maere@psb.vib-ugent.be).

Contributions

N.W., G.B., S.C.B., J.V., W.B, D.I., S.Ma. and H.N. conceived the original screening and research plans; D.I., S.Ma. and H.N. supervised the project. N.W. supervised the Phenovision experiments; L.V., N.W., S.Me. performed the experiments; L.V. and N.W. analyzed the organ growth data; L.V. analyzed the biomass data; B.C., K.M., K.D., J.D.B., J.M. and S.D. provided technical assistance during experiments; L.V., D.I., S.Ma. and H.N. wrote the article with input from the other authors. H.N. and S.Ma. agree to serve as the authors responsible for contact and ensure communication.

Abstract

Drought at flowering and grain filling greatly reduces maize (*Zea mays*) yield. Climate change is causing earlier and longer lasting periods of drought, which affect the growth of multiple maize organs throughout development. To study how long periods of water deficit impact the dynamic nature of growth, and to determine how these relate to reproductive drought, we employed a high-throughput phenotyping platform featuring precise irrigation, imaging systems, and image-based biomass estimations. Prolonged drought resulted in a reduction of growth rate of individual organs—though an extension of growth duration partially compensated for this—culminating in lower biomass and delayed flowering. However, long periods of drought did not affect the highly organized succession of maximal growth rates of the distinct organs, i.e., leaves, stems, and ears. Two drought treatments negatively affected distinct seed yield components: prolonged drought mainly reduced the number of spikelets, and drought during the reproductive period increased the anthesis-silking interval. The identification of these divergent biomass and yield components, which were affected by the shift in duration and intensity of drought, will facilitate trait-specific breeding towards future climate-resilient crops.

Introduction

As climate change confronts us with an uncertain future, the growing population ensures that humanity's need for nutrition will not decrease. It is clear that in order to keep improving crop yield per se and to safeguard crop yield under changing climatic conditions, continuous efforts in the field of plant breeding are necessary. Of all environmental constraints, water deficiency is the most prevalent abiotic stress in an agricultural context (Boyer, 1982) and is forecasted to become even more threatening in future decades (Wallace, 2000; Lobell et al., 2014). The strongest yield losses in cereals due to drought are caused by drought periods occurring around flowering, severely affecting reproductive development, seed set and seed growth (Saini and Westgate, 1999; Boyer and Westgate, 2004). In the future, crops will likely face longer periods of water deficit, occurring earlier in the season (Lehner et al., 2017; Hari et al., 2020).

Crop performance is ultimately summarized in the form of yield, which is by definition a direct product of final crop biomass and the harvest index (Wallace et al., 1993; Hay, 1995). Crop biomass in itself is a result of the growth of the different plant organs. Growth is a complex and dynamic process regulated by interactions between genetic factors and the environment. Growth is also a reflection of intricate source-sink dynamics. A leaf for example, will initially be a sink competing for resources with other growing organs before transitioning into a source organ that feeds the growth of other non-photosynthetic organs. Abiotic stress can further affect growth dynamics, as exemplified by drought that compromises both source and sink strength, severely disrupting plant growth and, ultimately, yield (Rodrigues et al., 2019).

Experimentally, growth is often studied by way of single end-point measurements or at limited time points due to constraints on time and resources and the destructive nature of measurements (Dhondt et al., 2013). These methods lack the temporal resolution required to truly capture the dynamic nature of growth. Studies that follow growth over time are often limited to one organ, neglecting the relationships with other organs, and thereby failing to reach an integrated view on how the growth of different organs is organized to reach final plant architecture and biomass. These issues may be alleviated by the use of high-throughput plant phenotyping platforms, which are equipped with imaging sensors and precise automated irrigation systems, a technology with the potential to provide measurements of several organs at high temporal resolutions (Fahlgren et al., 2015). These platforms allow mild drought treatments more akin to situations encountered in the field (Claeys and Inzé, 2013; Lawlor, 2013; Rodrigues et al., 2019), with a gradual onset of drought leading to growth and yield reductions, but not so severe as to threaten plant survival.

Individual maize (*Zea mays*) leaves are often used as a model for organs that exhibit linear growth (Nelissen et al., 2012; Avramova et al., 2015; Nelissen et al., 2016), and it is shown that growth rate, as well as growth duration, determine final leaf length (Nelissen et al., 2018). In this study, we investigate the plant growth dynamics of several growing organs during maize development and describe the relationships between the growth of maize leaves, stem, ears, and the plant as a whole. In addition, we compare the responses to prolonged drought (starting at V5 during development, V5-Drought) with the effects of reproductive drought (V12-Drought), by standardization of watering treatments and using a phenotyping platform. A negative correlation between growth rate and growth duration was observed for most individual leaves under drought, as previously observed for seedling leaves (Nelissen et al., 2018), but also, the variation in the pattern of final leaf lengths along the plant longitudinal axis was determined by an intricate interplay between these two processes. Moreover, stem growth under V5-Drought also displayed a negative relationship between growth rate and growth duration. When the growth of organs (leaves, stem and ear) was considered relative to other organs, it became clear that there was a highly organized succession of maximal growth for

the different organs, and that drought affected the amplitude and period of the distinct peaks, but not the coordination among them. Although the acute drought responses in growing organs were similar for V5- and V12-Drought, both conditions affected distinct seed yield components. V5-Drought reduced the number of spikelets that formed, while V12-Drought maintained almost full seed yield potential but mainly affected the anthesis-silking interval (ASI), and thus the success of actual pollination. These data indicate that the predicted prolonged drought spells (represented by V5-Drought) will have a major impact on both biomass and seed yield, which necessitates adjusting trait-specific breeding to address yield losses caused by climate change.

Results

Phenovision facilitates maize phenotyping throughout development under drought

Phenovision is a conveyor-belt based automated plant phenotyping platform located in a climate-controlled greenhouse chamber with a capacity of 392 plants in individual holders (supplemental Fig. S1). The platform is equipped with three weighing-watering stations for irrigation based on the gravimetric soil water content as well as the application of different nutrient solutions.

Environmental sensors are installed at four locations above the growing stage of the platform to measure air temperature, air humidity and the amount of photosynthetically active radiation (PAR) at five-minute intervals. Plants were imaged daily using visual, thermal infrared and hyperspectral imaging systems to follow plant growth and physiology throughout development. In this study, we used Phenovision to analyze the effects of mild soil water deficit on the growth of the whole shoot and the individual organs in the stiff-stalk maize inbred line B104. B104 is frequently used as a model inbred because it is easily amenable to transformation (Frame et al., 2006; Anami et al., 2010; Coussens et al., 2012).

Four experiments were conducted in the periods March-May and September-November in the years 2015 and 2016. In the first experiment, the optimal severity of the drought treatments was determined in order to achieve a reduction in growth without entirely terminating growth. Plants started at a well-watered soil water potential of -10 kPa, close to field capacity. Control treatment plants remained at this well-watered soil water potential for the entire experiment. Mild drought (Mild) or severe drought (Drought) treated plants were switched to a lower soil water potential of -28kPa or -100kPa at two developmental timepoints, the V5-stage (on average at a plant age of 16.3 days after plant emergence (DAE), when the ligule of the fifth leaf was fully exposed from the sheath (Ritchie et al., 1993) and the plant was firmly established and in vegetative development (Fig. 1; supplemental Fig. S2)), or the V12-stage (at a mean plant age of 39.6 DAE, by which point the reproductive organs had been initiated and stem growth had started) (Fig. 1; supplemental Fig. S2) (supplemental materials and methods S1). Plants switched to V5-Drought were not watered for 7 days on average before the soil water content was sufficiently reduced, and plants switched to V12-Drought were not watered for 3 days on average (Fig. 1B). Plants remained at the reduced soil water content until the end of the experiment. For the second to fourth experiments, only the Control and Drought treatments were used. The V5-Drought and V12-Drought treatments allowed for insight into the effects of drought on plant growth at different developmental stages and during the growth of different organs. In all experiments, plants were followed over development from emergence until silking, undergoing daily imaging and observation of leaf emergence and development. A subset of plants was selected for leaf length measurements over time, and others were destructively sampled for biomass measurements, stem length measurements and ear growth measurements (supplemental materials and methods S2;S3). The growth of B104 plants under the different watering regimes is illustrated in Fig. 1 and supplemental movie S1.

For leaves and stems, drought reduces the growth rate and extends the duration of growth, which progressively delays development

To monitor the coordination of growth between distinct organ types and to assess the effect of drought on the different organ types, the rate and timing of growth and development were monitored for leaves, stem and ear (Fig. 2). In seedling leaves, it has been shown that final leaf length (FLL) results from the interplay between the leaf elongation rate (LER) and leaf elongation duration (LED), and that drought lowers the LER, which is partially compensated by a prolonged LED (Avramova et al., 2015; Nelissen et al., 2018). Here, we investigated the effect of drought throughout plant development and evaluated whether the growth of mature leaves was more tolerant to or more severely affected by seedling drought.

Already in the Control conditions, there was a negative correlation between LER and LED across developing leaves, as later leaf ranks displayed a progressively reduced LER concomitant with an increase in LED from 10.0 days for leaf 6 to 30.3 days for leaf 18 (Table 1; Fig. 3; Fig. 4A; supplemental table S1). At an individual leaf level, V5-Drought caused a reduction of maximal LER between 27.5% and 33.1% for leaves 9, 12 and 15, but not for leaf 18 (Table 1; Fig. 4B), while LED was increased between 18.9% and 35.6% for leaves 9, 12 and 15 under V5-Drought conditions, but not for leaf 18 (Table 1; Fig. 2; Fig. 4B; supplemental table S1). As FLL was negatively affected by V5-Drought in all observed leaves, the reduction of LER was not compensated by the increase in LED in leaves 9-15, so LER was more affected by V5-Drought than LED (Table 1).

The V12-Drought treatment (Fig. 4C) started late in leaf growth and affected the growth of leaf 18 more than that of leaf 15. Leaf 15 was close to the end of its growth at the start of the V12-Drought treatment (~39.6DAE) and responded by ending its growth earlier, leading to a perceived increase in LER with respect to Control conditions (Table 1; supplemental table S1). For leaf 18, V12-Drought caused a significant reduction in leaf 18 mean LER, while the LED was unaffected (Table 1; supplemental table S1).

These data indicate that the FLL, which is maximal for leaf 12 and progressively reduces for subsequent leaves, is the result of a negative correlation between LER and LED under Control conditions (Fig. 3), indicating that leaves progressively grow slower but for a longer time. When drought was imposed prior to the development of the leaves, as in V5-Drought, the LER was lowered and the LED prolonged even more. This effect progressively increased with leaf rank but was absent from the last-formed leaves.

Whole-stem elongation was approached using two methods, either by taking the height of node 20 as the stem length and fitting a beta-sigmoid growth function to this length over time (supplemental Fig. S3A; supplemental Fig. S4D-F) or by taking the smoothed sum of the growth rates of the separate stem fractions (Fig. 4D-F). Both methods indicated that the peak of stem elongation took place during the growth of internodes 12-17 under Control conditions, with a maximal elongation rate of 68.52 mm/day for internodes 12-14. The stem elongation rate steadily increased up to this peak but decreased rapidly afterwards (Fig. 4D; Table 2).

Both V5-Drought and V12-Drought treatments significantly reduced mean stem elongation rate, going from 35.90 mm/day in Control conditions to 26.87 mm/day and 31.12 mm/day in V5-Drought and V12-Drought conditions, respectively ($p < 0.05$; Fig. 4E; Table 2; supplemental table S2). When looking at the separate stem fractions, this significant reduction in mean elongation rate was only found in internodes 9-11 and 12-14 for V5-Drought treated plants ($p < 0.05$; Fig. 4E; Table 2;

supplemental table S2), coupled with a significant extension of elongation duration in internodes 12-14 for V5-Drought treated plants ($p < 0.05$; Fig. 2; Table 3; supplemental table S2). The V5-Drought treatment caused a significant reduction in final length of internodes 1-8, 9-11 and 12-14 ($p < 0.05$; Table 2; supplemental table S2). For internodes 9-11 and 12-14, the reduction in final length is explained by the reduction in mean elongation rate; for internodes 1-8 there was no change in elongation rate, but a trend toward shorter elongation duration, which can explain the reduced final length. V12-Drought caused a significant reduction in final length of only internodes 12-14 ($p < 0.05$; Table 2; supplemental table S2). We observed the same drought response in internodes 12-14 under V5-Drought conditions as in the leaves: a reduction in elongation rate combined with an increase in elongation duration that was insufficient to compensate in the final length (Table 1, Table 2, Table 3).

V5- and V12-Drought affect different aspects of reproductive development and seed yield

In maize, final yield is determined by the number of developed spikelets and the success of pollination and seed set for these spikelets. We observed ear and tassel development, important components of seed yield, in the different treatments, to determine how different drought treatments affect seed yield potential.

Reproductive development was delayed by both drought treatments, but the flowering phenology was more severely affected in V5-Drought compared with V12-Drought (Fig. 2; supplemental table S3). Tassel and husk leaf emergence were delayed by 8.0 and 8.9 days, respectively, for V5-Drought and by 1.3 and 2.7 days, respectively, for V12-Drought, compared with the Control treatment, while anthesis and silking were both delayed by 6.1 days for V5-Drought and 0.8 and 1.8 days, respectively, in the V12-Drought treatment, compared to Control conditions (supplemental table S3). Opposite to the V5-Drought, V12-Drought had a stronger effect on ear-related development (husk leaf emergence and silking) than on tassel-related development (tassel emergence and anthesis), resulting in a more severely affected ASI, which is an important factor determining pollination success and final grain yield. In V12-Drought, the ASI was 6.3 days compared to 5.4 days in the Control treatment, while under V5-Drought conditions, the ASI comprised 5.1 days and was not significantly different from the Control treatment (supplemental table S3).

Ear elongation was delayed in both drought treatments compared to the Control treatment, but more so in the V5-Drought treatment than in the V12-Drought treatment (Table 4; Fig. 4I; supplemental Fig. S4G; supplemental table S4). Taking the moment at which the ear elongation rate reached 0.1mm/h (or 2.4mm/day) as a threshold for the start of elongation, ears in the Control treatment had started elongation at 49.4 DAE (95% CI 48.9-49.9), while ears in the V5-Drought treatment had started elongating by 56.9 DAE (95% CI 55.6-58.0), 7.5 days after the Control treatment, while ears in the V12-Drought treatment reached the threshold at 51.0 DAE (95% CI 50.5-51.6), 1.6 days after the Control treatment.

The total number of spikelets per ear, calculated as the product of the number of spikelets per row and the number of rows per ear, was fully determined during the earliest stages of ear elongation, before the elongation rate reached 0.1 mm/h (or 2.4mm/day), in all treatments (Fig. 4J). Fewer ear rows and spikelets per row formed in the V5-Drought treatment compared to the Control treatment (Fig. 4G-H; Table 4). Under V5-Drought, the mean final number of ear rows dropped from 13.1 in Control conditions to 11.5 in V5-Drought conditions, and the mean number of spikelets per row was reduced from 51.4 in Control conditions to 45.8 in V5-Drought conditions ($p < 0.05$; Table 4; supplemental table S3). In V12-Drought conditions, there was no significant reduction in final number of ear rows, with an average of 12.8 rows, but there was a significant reduction in the final

number of spikelets per row, which decreased to 48.7 on average in V12-Drought conditions ($p < 0.05$; Table 4; Fig. 4G-H; supplemental table S3). The total number of spikelets per ear was highest under Control conditions, with 670.4 spikelets per ear on average, compared to an average of 625.6 spikelets per ear under V12-Drought and 528.9 spikelets per ear under V5-Drought ($p < 0.05$; Table 4; Fig. 4J; supplemental table S3).

Both drought treatments thus affected different components of seed yield, with V5-Drought having a strong effect on the total number of spikelets per ear, directly reducing the seed yield potential, and V12-Drought having an increased ASI, potentially reducing pollination efficiency and final kernel yield.

Shoot biomass accumulation responds quickly to drought

Shoot biomass accumulation was chosen as a representation of whole shoot growth, which is ultimately the summation of the growth of the individual organs. In order to non-destructively measure biomass accumulation, shoot biomass was predicted using RGB imaging. Linear models were trained on a dataset consisting of individual plants that were destructively sampled for biomass measurements throughout development (supplemental materials and methods S4). Above-ground fresh weight and above-ground dry weight were predicted based on the projected plant area (area of the image classified as plant) of the RGB images. Using leave-one-out cross-validation (LOOCV), the image-based fresh-weight prediction model achieved an out-of-bag (oob) R^2 value of 0.967 and a mean-absolute-percentage-error (MAPE) of 7.83%, while the dry-weight model achieved an oob R^2 value of 0.935 and a mean-absolute-percentage-error (MAPE) of 12.90% (Fig. 5; supplemental table S5). Final models were trained using all available data points. Further details regarding the differences between fresh and dry weight prediction and the prediction performance for different treatments can be found in supplemental result S1 (supplemental Fig. S5; S6; S7; S8 and supplemental Tables S6; S7) and supplemental discussion S1.

Using the image-based plant biomass estimation models, biomass was estimated over time and the daily biomass accumulation rate was calculated (Fig. 6). The V5-Drought treatment had a strong and rapid negative effect on the plant biomass accumulation rate (Fig. 6C&D; supplemental table S8;S9), which was significant within a day after the start of the V5-Drought treatment (16.3 DAE) ($p < 0.05$, Mann-Whitney U test, Holm-Bonferroni multiple testing correction). As such, V5-Drought treated plants displayed a significant reduction in fresh and dry weights compared to the Control treatment, from 19 DAE until 61 DAE (Fig. 6A&B; supplemental Table S10;S11). This reduction in biomass accumulation rate remained significant until at least 39 DAE for fresh weight. From 40 DAE onward until about 45 DAE, biomass accumulation could not be estimated accurately because stem widening caused the lower leaves to detach from the plant in all treatments. These leaves blocked the phenotyping platform and had to be removed, leading to a reduction in plant biomass and causing unreliable plant biomass increase rate inferences. For Control treated plants, this reduction was estimated to be between 18 g and 20 g of FW, occurring mainly between 40-42 DAE, leading to a perceived drop of the FW biomass accumulation rate by about 6-7 g/day during these days. Dips and peaks coincided on the same days for the different treatments, as these leaves were manually removed on particular days for all treatments at the same time. The dry weight accumulation rate in the V5-Drought treatment was significantly reduced from 17 DAE until 51 DAE. In this case, the treatment effect is stronger than the noise caused by the removal of platform-obstructing leaves.

For V12-Drought treated plants, there was a significantly lower fresh weight and dry weight compared to Control starting from 41 DAE and 42 DAE, respectively, which was shortly after the start of V12-Drought treatment (39.6 DAE) until 61 DAE (Fig. 6A&B; supplemental Table S10;S11). The

start of the V12-Drought coincided with the detaching of the lower leaves, meaning that biomass accumulation rate was determined less accurately in the beginning. However, the fresh weight accumulation rate was significantly reduced at 41, 42, 44 and 45 DAE, and the dry weight accumulation rate was significantly reduced from 41-45 DAE, at 47-48 DAE and at 54 DAE in the V12-Drought treatment compared to the Control treatment (Fig. 6C&D; supplemental table S8;S9).

The effects of drought on shoot growth were immediately reflected in the biomass accumulation rate, and were most visible during the periods when biomass accumulation rate was rising and shortly after its peak (Fig. 6C&D; supplemental table S8;S9). The biomass accumulation rate dropped earlier in the Control treatment than in both drought treatments, likely due to the developmental delay caused by drought, causing the difference between Control and both drought treatments to diminish (Fig. 6C&D; supplemental table S8;S9).

Drought reduces leaf, stem and ear growth to a similar extent but does not affect the succession of growth across the different organs

To compare the growth of the different organs and the biomass accumulation, the growth rate variables were divided by their maximum value under control conditions, resulting in normalized growth rates in a range from 0 to 1 (Fig. 7; supplemental Fig. S9). Comparing the distinct organs revealed a clear succession in growth of the different organs, which was maintained in all three treatments. First, leaf growth culminated around the time stem growth started. Then leaf growth decreased synchronously with the increase in stem growth, which reached its maximum close to the end of leaf growth. Stem growth then decreased rapidly as ear growth increased (Fig. 7). Fresh weight accumulation peaked during the transition from leaf to stem growth. Dry weight accumulation seemed to follow fresh weight accumulation with a delay, suggesting a closer association with stem growth than leaf growth (supplemental Fig. S9A).

V5-Drought had immediate and distinct effects on the leaf growth rate and the biomass accumulation rate. When the drought treatment started, the leaf growth rate stopped increasing and peaked at 82% of the maximum leaf growth rate of the Control treatment (Fig. 7; supplemental Fig. S9B). The fresh weight accumulation rate showed a temporary setback immediately after the onset of the drought treatment. After 4 days of progressive reduction, the fresh weight accumulation rate started increasing again, finally reaching 62% of the maximum growth rate in the Control treatment (supplemental Fig. S9). Stem growth was delayed and reduced under V5-Drought, peaking at 81% of the maximal growth rate under Control conditions, which was very close to the reduction observed for leaf growth rate (Fig. 7; supplemental Fig. S9B). The maximum observed growth rate of the ear was 73% of the maximum rate of the Control treatment (Fig. 7; supplemental Fig. S9B). Fresh and dry weight accumulation rates were more impacted by V5 drought than were the growth rates of the individual organs, as fresh weight and dry weight accumulation rates peaked at 62% and 52%, respectively, of the maximal accumulation rate under Control conditions (supplemental Fig. S9). This makes sense, since the measured organ elongation rates are linear (1D), whereas fresh and dry weight accumulation rates demonstrate 3D growth. If leaf width, thickness and length, for instance, were similarly affected by V5-Drought, the expected peak leaf biomass accumulation rate would be $0.82^3 = 55\%$ of the maximum rate under Control conditions, which was in line with our observations (supplemental Fig. S9). Leaf growth was extended in time and stem growth was delayed, causing the peak in fresh weight accumulation to be delayed as well (Fig. 7; supplemental Fig. S9).

The V12-Drought treatment caused the dry weight accumulation rate to stop increasing and peak at 86% of the maximum rate under Control conditions (supplemental Fig. S9). The fresh weight accumulation rate quickly dropped to around 52% of the maximum rate of Control conditions

between 45 and 48 DAE, similar to the rate for plants in the V5-Drought treatment at the same age (supplemental Fig. S9B). The stem growth rate was reduced to 87% of the maximum growth rate under Control conditions, but there was no delay in stem growth in this treatment. There was, however, a delay in ear growth, along with a reduction in observed growth rate, to 74% of the maximum growth rate under Control conditions, which was similar to the reduction observed in the V5-Drought treatment (Fig. 7; supplemental Fig. S9B).

In summary, organs grew in a clear succession in all treatments and growth of each organ in the sequence consistently diminished with increasing growth of the next organ. Drought was observed to have a rapid effect on organ growth rates and biomass accumulation rates, and both the V5- and V12-Drought treatments had a similar effect on growth rates.

Discussion

Mild drought extends organ and plant growth

The effects of drought at both organ level and whole shoot level show a recurrent pattern: a reduction in growth rate that is partially compensated by an extension in growth duration. This compensation phenomenon has been described previously in maize leaves (Avramova et al., 2015; Avramova et al., 2017; Nelissen et al., 2018) and is thought to maintain the capacity to resume growth for a longer period in anticipation of water becoming available again. It is also possible that the plant has a structured plan for its development and when one mechanism is perturbed, the organism will attempt to maintain final organ size by compensating through other mechanisms (Hisanaga et al., 2015). Either way, tracking the plants' entire development reveals a downside of this strategy in the long run. As a maize plant cannot support too many concurrently growing organs (Fig. 7), extending the growth of the earlier organs inevitably delays the start of growth of the later organs. This ultimately extends the time needed for the plant to finish its developmental cycle. In this study, plants under well-watered conditions required on average 61 days to reach silking, in contrast to plants in the V5-Drought treatment that reached silking after 67 days on average. A similar effect was seen in Sun et al. (2017), where overexpression of the maize *PLASTOCHRON1* (*ZmPLA1*) gene led to increased final leaf sizes by increasing the LEDs, but also delayed silking by 6 days, up to 72 days after sowing in the transgenic plants compared to 66 days after sowing for non-transgenic plants (Sun et al., 2017). This extension of plant development can pose a problem in conditions where the growing season is limited in time, as delayed development increases the risk of seed set being affected by adverse conditions and further reducing yield (Kenny and Harrison, 1992; Harrison et al., 2014).

An interesting result of the drought-induced delay in development was observed in the comparison of the ASI between V5-Drought treated plants and V12-Drought treated plants. Even though silking was delayed in both treatments compared to the Control treatment, the ASI was affected only in the V12-Drought treatment. This might be due to a disconnect in the severity by which tassel and ear development were affected in the V12-Drought treatment. The ear was still developing at the time the V12-Drought started. At that stage, the tassel is already further in its development (Siemer et al., 1969), and thus anthesis is less delayed by the V12-Drought. Long ASIs are known to negatively affect yield by reducing pollination success, as a higher fraction of the pollen has been shed by the time the silks emerge, and the exposure of the silks to viable pollen is reduced (Hall et al., 1982; Struik et al., 1986; Edmeades et al., 1993). Despite plants experiencing the same soil water content at the reproductive stage in both V5-Drought and V12-Drought, there is a clear difference in impact depending on whether drought is first sensed at that stage or whether an earlier, prolonged drought has persisted to this stage.

The highly coordinated succession of growing organs accompanies changes in source-sink interactions

Most of the plant organs form and grow post-embryonically. Leaves are formed at the flanks of the shoot apical meristem with a well-organized spacing (phyllotaxy) and timing (plastochron) (Ritchie et al., 1993; Freeling and Walbot, 1994). Here, we show that the growth of distinct organs, even when arising from distinct meristems such as the shoot-apical-meristem (SAM) and intercalary meristem (IM), is also highly coordinated.

In maize, initial growth is concentrated in the leaves. The overall leaf growth rate peaks around the time stem elongation starts, during the elongation of the earlier leaves. Later leaves have a progressively lower elongation rate while the stem growth rate increases. Finally, the ear growth rate rises when stem growth is declining. The highest biomass accumulation rate is achieved when leaf growth is declining and stem growth is not yet maximal. The highly organized succession of organ growth suggests the presence of signaling pathways between distinct growing organs and meristems. The nature of the signal involved is currently not known.

The growth of individual leaves and stem fractions, but also the cumulative growth of leaves and stem, as well as the shoot biomass accumulation, are characterized by sigmoid growth curves, with an early accelerating phase, a linear phase and a saturation phase. However, the specifics of the sigmoid growth curves, such as the slope of the linear phase (maximum growth rate) and the duration of the distinct phases, differed within and between organ types. Throughout leaf development, we saw a marked change in growth behavior from earlier leaves to later leaves. Leaves 6-9 were characterized by a shorter duration of leaf elongation, but the maximum leaf elongation rate increased with leaf number. Leaves 12-18 had a progressively lower leaf elongation rate but the duration of leaf elongation increased. The tipping point from increasing LER to decreasing LER coincided with the start of stem growth. The negative developmental correlation between LER and LED shows that growth rate and duration are tightly coordinated to determine final organ size.

The drought treatments also impacted growth processes. They lowered the growth rate and lengthened the growth duration of plant organs. The fact that lower LER and higher LED are observed in both older leaves under normal conditions and drought-stressed leaves suggests that similar mechanisms of resource limitation might be at work. Further indication for the similarity of these mechanisms is that if one mechanism is active, activating the other has little additional effect, as indicated by the reduced effect of drought treatment on the top leaves, represented by leaf 18 in this study. As resources for the top leaves already appear to be limited under Control conditions, there is little further limitation drought conditions can impose on these leaves without completely halting growth. In drought conditions, the lower LER is a consequence of a reduced supply of resources to the growing leaf, which is directly due to reduced source strength in the form of reduced carbon assimilation in mature leaves (Pastenes et al., 2005; Dias and Brüggeman, 2010; Jedmowski et al., 2014; Gururani et al., 2015), a greater negative water potential reducing the turgor pressure necessary for cell expansion (Passioura and Fry, 1992), and indirectly due to a reduced sink strength caused by a drought-induced downregulation of genes and proteins involved in cell wall biosynthesis and the cell cycle (Cramer et al., 2013; Avramova et al., 2015; Bechtold et al., 2016). In the case of the growing higher leaves, there is no indication of a reduced source strength. It is possible that the developing stem gradually increases its sink strength, which might be combined with a reduction in leaf sink strength, increasingly drawing resources to stem growth that were previously directed towards leaf growth. The transition from stem growth to ear growth displays a similar pattern, with a reduced growth rate for the upper internodes, which are growing during a period when ear growth is increasing. The increasing sink strength of the ear likely draws resources away from stem growth.

The effect of drought on the organ growth rates can be further generalized to state that drought affects fast-growing organs more than slow-growing organs, as the fast-growing organs appear to be stronger resource sinks and are more affected by disruptions to source strength. Source-sink interactions, the changes in these interactions throughout development and under stress, and the molecular regulation hereof play a crucial role in determining final plant yield, but many questions remain on how these intricate balances can be improved in an agricultural setting (White et al., 2016; Chang and Zhu, 2017)

Application of phenotyping platforms

Further advances in our understanding of plant growth require understanding of all factors involved, including genetics, environment, physiology, morphology and more. Phenotyping platforms such as the Phenovision platform employed in this study can help elevate phenotype profiling to the same level as molecular profiling, which is necessary to maximally reap the benefits from the integration of these different data types. We documented the ability of the Phenovision to follow whole shoot growth non-destructively over time and to quantify the effects of drought on shoot growth. By combining the platform's ability to non-destructively estimate shoot biomass with measurements of organ growth, we have been able to gain insights into the relations between the growth of different organs over development in maize. Irrigation systems such as the one used here could also be used to supply nutrient solutions and study the effects of various nutritional deficits. We limited ourselves to one genotype, but the platform could easily be used to investigate effects at genotype level and interactions between genotype and treatment, to assess genetic effects on drought and nutrient deficit tolerance.

Imaging systems are not limited to measuring whole shoot growth. With advances in the computer vision and machine learning fields, the potential of images for non-destructive measurements keeps increasing, and e.g., also allows the tracking of growth at the organ level (Brichet et al., 2017). The use of image analysis need not be limited to expensive automated phenotyping platforms. Raspberry Pi-based imaging setups are low-cost options for acquiring images (Tovar et al., 2018) that, combined with sufficient training data, can be used for the same non-destructive shoot biomass predictions as in this study. In addition to RGB imaging systems for measuring growth, platforms can also be equipped with hyperspectral and thermal infra-red imaging systems to non-destructively monitor plant physiological status and drought response (Asaari et al., 2019). Automatically gathered information on plant structure and physiology can also form the basis for functional-structural plant models (Vos et al., 2010; Liu et al., 2019), which if combined with molecular information, can be a powerful tool in systems biology to integrate all relevant aspects of plant growth and yield.

The observation that the climate change-induced shift in the duration of drought might affect yield components distinct from reproductive drought means that the attention should also be refocused towards different or additional traits in future breeding, such as resource partitioning during early drought. For example, we found an effect of the V5-Drought on the amount of spikelets per ear, which would seriously hamper the yield potential of the plants even if environmental conditions would improve during seed filling. A possible focus for future research could be to work on plants that maintain the number of spikelets per ear when confronted with adverse conditions early on. One could imagine a plant that prioritizes ear development over stem growth in resource-limited conditions such as drought. Given the increasing frequency of prolonged drought spells in many areas of major crop production (Toresi et al., 2019; Hari et al., 2020), it is necessary for these additional traits to be improved soon. New breeding techniques that allow for more targeted and multiplex approaches to address these multigenic traits in a relative short time span will be pivotal to obtain climate-resilient crops that can cope with longer drought spells in the near future. In addition,

integrative image analysis following several organs simultaneously combined with spectral imaging will provide more insights into the coordination of distinct organs during drought stress.

Materials and methods

This section contains abbreviated materials and methods, full descriptions of the methods used can be found in the supplemental materials and methods. R-scripts and data related to the biomass prediction models have been made available through Zenodo (<https://doi.org/10.5281/zenodo.4323615>).

Experimental setup

Experiments were conducted in the Phenovision high-throughput plant phenotyping platform (supplemental Fig. S1; supplemental materials and methods S5). Plants were grown in a semi-controlled greenhouse setting (supplemental materials and methods S6). Plants were all of the stiff-stalk maize (*Zea mays* L.) inbred line B104, sown individually in 7 l pots with approximately 850 g (dry weight) of peat-based soil enriched with osmocote slow-release fertilizer (N.V. Van Israel, Belgium). All plants were watered daily up to their target gravimetric soil water content (supplemental materials and methods S1; supplemental Fig. S10). All plants started the experiment at the well-watered target soil water content. Drought-treated plants were switched to a lower target soil water content upon reaching either the V5 or V12 stage, and maintained this lower target soil water content until the end of the experiment. Four experiments were conducted in the periods March-May and September-November in the years 2015 and 2016. In the first experiment, 250 plants were destructively sampled throughout development in order to measure fresh and dry weights, stem growth, and ear development (supplemental materials and methods S2; supplemental data S1; S2). Subsets of plants were also destructively sampled for phenotyping in the second to fourth experiments (supplemental materials and methods S2; supplemental data S2). In all experiments, plants were visually inspected daily to check their developmental stage (supplemental materials and methods S3; supplemental data S3). In the first experiment, the length of leaf 6 was measured during its growth for all plants, and 100 plants were selected for leaf length measurements throughout the growth of leaves 9, 12, 15 and 18 (supplemental materials and methods S3). Additionally, leaf 4 lengths were tracked for 12 Control treated plants during a smaller trial in February 2015.

Organ growth analysis

Leaf growth was analyzed by fitting beta-sigmoid growth curves and three-piece-linear growth curves to the leaf length time series of individual leaves in R version 3.6.3 (R Core Team, 2020) in order to determine LER over time, LED, and FLL (supplemental materials and methods S7). Stem length was measured destructively, thus there were no time series for individual stems; instead beta-sigmoid and three-piece linear growth curves were fitted per treatment (supplemental materials and methods S8). Estimates of the elongation rate over time, elongation duration and final length were determined per treatment from these growth curves. A bootstrap resampling method was used to determine 95% confidence interval (CI) for the derived stem traits. The length of ears sampled throughout ear development until silking was determined together with the number of ear rows and the number of spikelets per row (supplemental materials and methods S9; supplemental data S2; S4). Since the sampling concluded at silking, only the first part of ear elongation was observed. Therefore, it was not possible to fit a sigmoid growth curve, and instead, an exponential growth curve was fitted to the ear length data per treatment over time. The ear elongation rate was derived from this curve, and the moment at which the elongation rate reached 0.1 mm/h (or 2.4 mm/day) was taken as a threshold to compare the start of ear growth between treatments. A bootstrap

resampling method was used to determine 95% CI for this moment in order to allow statistical analysis (supplemental materials and methods S9).

RGB image analysis and biomass prediction

Plants were imaged daily using the RGB imaging system, which captured 1 top-view picture and 6 sideview pictures of the plants at different angles, resulting in an almost hemispheric view (supplemental materials and methods S10; supplemental Fig. S11A). Plant pixels on the images were identified using support vector machine classification, and the number of pixels was then converted to projected plant area (supplemental materials and methods S10; supplemental Fig. S11). Linear models to predict shoot fresh weight and shoot dry weight from the projected plant area values were trained using all available destructive biomass measurements from the first experiment (supplemental materials and methods S4; supplemental table S12; supplemental data S5; supplemental results S1 and supplemental discussion S1). Plant fresh and dry weights were then predicted for the plants in the second, third and fourth experiments using the projected plant area values derived from the daily images. Fresh and dry weight accumulation rates were determined from the differences between predictions on subsequent days. The biomass and biomass accumulation rates were compared between different treatments on a day-by-day basis using Mann-Whitney U tests followed by multiple-testing correction using the Holm-Bonferroni method (Holm, 1979).

Statistical analysis

Analyses were performed in R version 3.6.3 (R Core Team, 2020), and data were plotted using the package ggplot2 (Wickham, 2016).

Accession Numbers

Sequence data from this article can be found at www.maizegdb.org under accession number GRMZM2G167986 (PLA1).

Supplemental data

The following supplemental materials and methods, results, discussion, figures, movie, tables and data are available.

Supplemental Materials and Methods S1-S10

Supplemental Method S1. Watering.

Supplemental Method S2. Destructive sampling.

Supplemental Method S3. Repeated observations and measurements.

Supplemental Method S4. Biomass prediction model.

Supplemental Method S5. Experimental setup.

Supplemental Method S6. Environment.

Supplemental Method S7. Leaf growth analysis.

Supplemental Method S8. Stem growth analysis.

Supplemental Method S9. Ear growth and development.

Supplemental Method S10. RGB imaging setup and plant pixel segmentation.

Supplemental Results S1. Treatment specific biomass modeling

Supplemental Discussion S1. Biomass modeling

Supplemental Figure S1. Overview of the Phenovision platform.

Supplemental Figure S2. Distribution of plant age upon reaching the V5-stage for Control, V5-Drought and V12-Drought treatments.

Supplemental Figure S3. Beta-sigmoid and three-piece-linear growth functions fitted to stem fraction length data and leaf length data and exponential growth function fitted to ear length data.

Supplemental Figure S4. Length data used for fitting growth curves of leaves, stems, stem fractions and ears.

Supplemental Figure S5. Relation between shoot biomass and projected plant area.

Supplemental Figure S6. Prediction performance using LOOCV of treatment specific image-based fresh weight prediction models.

Supplemental Figure S7. Comparisons of water content and stem/leaf ratio of fully grown plants in different watering treatments.

Supplemental Figure S8. Relation between plant projected sideview and top-view area, plant age and predicted fresh weight.

Supplemental Figure S9. Normalized organ growth rates and biomass accumulation rates over time.

Supplemental Figure S10. Soil water retention curve, showing the relation between soil water potential and gravimetric water content for the potting soil used.

Supplemental Figure S11 Illustration of plant RGB imaging angles and plant pixel segmentation

Supplemental Table S1. Detailed results of the leaf growth analysis.

Supplemental Table S2. Detailed results of the stem growth analysis.

Supplemental Table S3. Detailed results of the statistical analysis of ear and tassel development traits.

Supplemental Table S4. Detailed results of the ear growth analysis.

Supplemental Table S5. Overview of biomass prediction models performance parameters.

Supplemental Table S6. Coefficients of treatment-specific linear fresh weight prediction models for the model featuring only sideview plant projected area and the model featuring sideview and top-view plant projected area as well as the interaction term

Supplemental Table S7. Overview of treatment-specific fresh weight prediction models performance parameters

Supplemental Table S8. Shoot fresh weight accumulation rate over time .

Supplemental Table S9. Shoot dry weight accumulation rate over time.

Supplemental Table S10. Shoot fresh weight over time.

Supplemental Table S11. Shoot dry weight over time.

Supplemental Table S12. Overview of formulas used to calculate model performance parameters

Supplemental Movie S1. Plant growth and development over time on the Phenovision platform for different watering treatments.

Supplemental Data S1. Destructive biomass and node height sampling data gathered in experiment 1.

Supplemental Data S2. Raw ear sampling data

Supplemental Data S3. Ontogeny data

Supplemental Data S4: Raw ear sampling data for fully developed ears .

Supplemental Data S5: Image-based biomass estimation raw data and predictions for experiment 1

Acknowledgements

This work was supported by the Hercules Foundation (ZW1101) and the 'Bijzonder Onderzoeksfonds Methusalem Project' (no. BOFMET2015000201) of Ghent University.

Tables

Table 1 Overview of leaf growth traits, showing the mean plant age at the start and end of leaf growth in DAE, mean plant age at leaf emergence from the whorl, LED (days), maximal LER (mm/day), mean LER after leaf emergence (mm/day) and FLL (mm). Deviations of start and end of leaf growth and leaf emergence in V5- or V12-Drought conditions relative to Control conditions are given in days, for other variables relative values are indicated as % of Control values. Significant differences are indicated by * ($p < 0.05$, two-sample t-test if conditions were met, otherwise non-parametric Wilcoxon-Mann-Whitney test). Full details and numbers of samples (n) are available in Supplemental Table S1.

leaf	treatment	start (DAE)	relative to control (days)	plant age at leaf emergence (DAE)	relative to control (days)	end (DAE)	relative to control (days)	LED (days)	LED (% of control)	max LER (mm/day)	max LER (% of control)	mean LER (mm/day)	mean LER (% of control)	FLL (mm)	FLL (% of control)
6	Control	9.0		11.7		19.0		10.0		101.03		92,02		921.8	
9	Control	13.7		17.4		27.0		13.2		98.06		91,07		1207.4	
9	V5-Drought	13.7		17.4	0.0	29.5	2.5 *	15.7	118.9 *	70.34	71.7 *	64,65	71.0 *	1144.7	94.8 *
12	Control	17.6		23.6		35.1		17.4		75.95		70,46		1228.3	
12	V5-Drought	17.5	-0.1	26.2	2.6 *	39.7	4.6 *	22.1	126.8 *	54.73	72.1 *	51,11	72.5 *	1132.2	92.2 *
15	Control	21.4		30.4		43.1		21.7		56.76		52,70		1116.3	
15	V5-Drought	20.4	-1.1	34.2	3.8 *	49.7	6.6 *	29.4	135.6 *	37.98	66.9 *	35,35	67.1 *	1056.0	94.6 *
15	V12-Drought	21.4		30.4	0.0	42.0	-1.0 *	20.6	95.2	63.31	111.5 *	58,96	111.9	1079.2	96.7
18	Control	22.4		37.4		52.7		30.3		31.98		29,48		914.3	
18	V5-Drought	28.0	5.6 *	43.3	5.9 *	56.8	4.1 *	28.8	94.9	32.07	100.3	28,83	97.8	803.6	87.9 *
18	V12-Drought	22.4		37.4	0.0	52.6	-0.2	30.1	99.4	28.20	88.2	24,83	84.2 *	847.4	92.7 *

Table 2 Maximal and mean elongation rates and final length of different stem fractions. Numbers in bold indicate the estimated values, and numbers in parentheses indicate the lower and upper boundaries of the 95% confidence intervals (CIs). Trait differences from the Control treatment and the associated 95% CI are also shown for the drought treatments. Significant differences are indicated with * ($p < 0.05$, based on the 95% CI of the difference, see Supplemental Method S8). N= 48 for the Control treatment, n=49 for the V5-Drought treatment and n=37 for the V12-Drought treatment (22 V12-Drought samples taken after V12-stage, complemented with data from 15 Control-treated plants sampled before the V12-stage). Full details are available in Supplemental Table S2.

Stem fraction	treatment	Maximal elongation rate (mm/day) + 95%CI	relative to control (mm/day) + 95 %CI	Mean elongation rate (mm/day) + 95%CI	relative to control (mm/day) + 95 %CI	Final length (mm) + 95%CI	relative to control (mm) + 95 %CI
1-8	Control	18.14 (0.00; 29.45)		11.48 (11.48; 21.15)		170.2 (161.1; 179.3)	
1-8	V5-Drought	18.27 (11.96; 72.46)	0.13 (-13.44; 58.04)	12.02 (12.02; 37.59)	1.43 (-5.72; 11.14)	118.3 (113.5; 123.8)	-51.9 (-62.3; -41.7) *
1-8	V12-Drought	17.95 (0.00; 94.76)	-0.19 (-20.70; 72.27)	14.99 (14.99; 1748.21)	-0.59 (-7.56; 5.95)	165.8 (155.6; 179.0)	-4.4 (-18.4; 11.2)
9-11	Control	35.75 (24.11; 50.99)		18.67 (18.67; 38.40)		285.3 (273.1; 296.8)	
9-11	V5-Drought	23.10 (19.99; 27.20)	-12.65 (-31.33; -2.76) *	15.36 (15.36; 27.85)	-10.38 (-24.13; -1.24) *	220.3 (208.8; 230.9)	-65.0 (-80.4; -48.5) *
9-11	V12-Drought	36.22 (0.00; 52.03)	0.47 (-22.96; 24.13)	17.94 (17.94; 40.33)	-1.07 (-19.27; 17.88)	290.7 (275.0; 326.8)	5.4 (-15.5; 35.9)
12-14	Control	68.52 (0.00; 102.21)		35.85 (35.85; 104.63)		335.4 (317.5; 349.3)	
12-14	V5-Drought	33.83 (23.94; 56.63)	-34.69 (-82.95; 10.16)	17.93 (17.93; 28.85)	-43.07 (-89.00; -16.82) *	233.9 (210.6; 252.2)	-101.5 (-128.4; -76.7) *
12-14	V12-Drought	79.05 (65.81; 93.37)	10.53 (-26.46; 78.64)	48.25 (48.25; 50.25)	-16.55 (-60.94; 8.76)	251.7 (240.1; 259.6)	-83.8 (-101.6; -65.9) *
15-17	Control	47.62 (0.00; 73.61)		26.54 (26.54; 60.00)		329.6 (289.1; 364.0)	
15-17	V5-Drought	37.49 (12.13; 51.12)	-10.13 (-66.61; 18.60)	12.17 (12.17; 36.40)	-8.47 (-38.98; 5.21)	312.3 (285.4; 373.1)	-17.3 (-63.4; 52.2)
15-17	V12-Drought	41.99 (29.96; 51.83)	-5.63 (-38.18; 151.15)	22.74 (22.74; 38.41)	-8.13 (-30.49; 4.91)	308.0 (287.7; 321.8)	-21.6 (-61.0; 24.7)
18-19	Control	39.99 (-2621.75; 81.43)		19.42 (19.42; 306.79)		279.4 (189.0; 2976.7)	
18-19	V5-Drought	31.86 (0.00; 106.25)	-8.13 (-141.59; 5185.71)	8.50 (8.50; 74.17)	-0.13 (-55.50; 49.89)	208.3 (189.8; 620.6)	-71.1 (-2488.9; 60.6)
18-19	V12-Drought	40.49 (0.00; 106.49)	0.50 (-124.38; 367.44)	10.01 (10.01; 33.98)	-0.44 (-126.82; 10.91)	231.2 (215.4; 248.5)	-48.2 (-2453.7; 52.7)
Stem	Control	47.10 (41.35; 57.89)		35.90 (35.90; 42.48)		1369.9 (1161.8; 1653.5)	
Stem	V5-Drought	37.92 (31.70; 42.69)	-9.18 (-22.44; -0.53) *	26.87 (26.87; 35.50)	-8.33 (-13.99; -2.33) *	1069.4 (990.6; 1422.7)	-300.5 (-621.3; 14.0)
Stem	V12-Drought	41.02 (36.23; 46.23)	-6.08 (-18.72; 2.26)	31.12 (31.12; 37.42)	-4.91 (-10.75; -0.33) *	1191.4 (1151.5; 1238.2)	-178.5 (-478.5; 37.1)

Table 3 Growth start, end and duration traits for different stem fractions and the entire stem. Numbers in bold indicate the estimated values, numbers in parentheses indicate the lower and upper boundaries of the 95% confidence intervals (CI). Trait differences from the Control treatment and the associated 95% CI are also shown for the drought treatments. Significant differences are indicated with * ($p < 0.05$, based on the 95% CI of the difference, see Supplemental Method S8). $n = 48$ for the Control treatment, $n = 49$ for the V5-Drought treatment and $n = 37$ for the V12-Drought treatment (22 V12-Drought samples taken after V12-stage, complemented with data from 15 Control-treated plants sampled before the V12-stage). Full details are available in Supplemental Table S2.

stem fraction	treatment	Age at start of growth (DAE) + 95%CI	delay relative to control (days) + 95 %CI	Age at end of growth (DAE) + 95%CI	delay relative to control (days) + 95 %CI	Duration of growth (days) + 95%CI	relative to control (days) + 95 %CI
1-8	Control	24.1 (19.8; 26.0)		35.0 (30.4; 35.8)		10.9 (7.9; 14.4)	
1-8	V5-Drought	30.3 (28.8; 32.6)	6.2 (3.4; 10.1) *	37.2 (35.6; 38.7)	2.2 (1.0; 4.5) *	6.9 (3.0; 9.8)	-4.0 (-8.8; 0.4)
1-8	V12-Drought	24.0 (23.9; 31.0)	-0.1 (-3.8; 4.3)	35.0 (30.0; 35.0)	0.0 (-2.4; 1.4)	11.0 (0.1; 11.0)	0.1 (-5.4; 5.2)
9-11	Control	31.7 (29.4; 33.2)		42.2 (40.4; 45.0)		10.5 (7.3; 15.2)	
9-11	V5-Drought	36.0 (35.2; 38.8)	4.3 (2.5; 7.4) *	49.0 (45.7; 49.2)	6.8 (4.4; 8.8) *	13.0 (7.7; 14.1)	2.5 (-2.3; 6.1)
9-11	V12-Drought	31.6 (29.2; 33.2)	-0.2 (-2.7; 2.3)	42.7 (40.4; 45.2)	0.5 (-2.6; 3.6)	11.1 (7.1; 15.5)	0.6 (-4.6; 5.9)
12-14	Control	44.4 (41.2; 45.2)		49.5 (48.4; 50.8)		5.1 (3.1; 8.7)	
12-14	V5-Drought	45.3 (44.4; 46.7)	0.9 (-0.4; 3.6)	55.8 (54.2; 58.0)	6.3 (3.9; 8.6) *	10.5 (8.0; 13.3)	5.4 (1.3; 8.9) *
12-14	V12-Drought	44.8 (44.8; 44.8)	0.4 (-0.5; 3.3)	49.9 (49.6; 50.1)	0.4 (-0.5; 1.8)	5.1 (4.9; 5.4)	0.0 (-3.0; 2.2)
15-17	Control	47.5 (46.1; 48.8)		56.7 (53.7; 59.6)		9.1 (5.2; 12.9)	
15-17	V5-Drought	53.4 (51.8; 54.2)	5.9 (3.9; 7.4) *	64.8 (62.0; 73.4)	8.1 (4.4; 14.1) *	11.4 (8.1; 25.0)	2.2 (-2.4; 9.7)
15-17	V12-Drought	48.4 (47.8; 49.8)	0.9 (-0.6; 2.7)	59.4 (57.3; 61.5)	2.7 (-2.0; 6.2)	11.0 (7.9; 13.6)	1.8 (-3.6; 6.4)
18-19	Control	54.6 (53.5; 61.0)		64.6 (60.8; 68.4)		10.0 (1.7; 13.7)	
18-19	V5-Drought	60.2 (55.1; 62.4)	5.6 (-2.7; 8.6)	67.9 (63.7; 73.3)	3.4 (-3.0; 10.4)	7.8 (1.8; 23.9)	-2.3 (-10.5; 13.6)
18-19	V12-Drought	55.3 (53.8; 59.4)	0.8 (-4.9; 2.8)	63.9 (62.8; 75.0)	-0.7 (-4.3; 9.9)	8.6 (6.8; 23.0)	-1.5 (-6.5; 13.7)
Stem	Control	28.5 (27.4; 29.6)		64.9 (63.5; 67.4)		36.3 (34.4; 39.8)	
Stem	V5-Drought	34.4 (32.4; 36.5)	5.9 (3.5; 8.4) *	69.7 (65.4; 76.0)	4.9 (-0.1; 10.0)	35.3 (30.0; 42.3)	-1.0 (-7.8; 5.5)
Stem	V12-Drought	27.5 (26.4; 28.6)	-1.0 (-3.0; 0.6)	62.6 (60.0; 64.0)	-2.3 (-5.7; 0.8)	35.1 (31.8; 37.6)	-1.3 (-5.7; 3.1)

Table 4 Overview of ear traits. For the mean number of ear rows, mean number of spikelets per row and mean number of spikelets per ear, drought values relative to control are also shown as percentages. The age at the start of growth was determined as the age at which the elongation rate reaches 0.1mm/h (or 2.4 mm/day). The estimate is indicated in bold and the 95% confidence interval is indicated in parentheses. The absolute delay relative to the Control treatment is indicated in days for the drought treatments. Significant differences are marked with * ($p < 0.05$, two-sample t-test if conditions were met, otherwise non-parametric Wilcoxon-Mann-Whitney test for number of ear rows, number of spikelets per row and number of spikelets per ear, based on the 95% CI for the age at start of growth). Full details and sample numbers (n) are available in Supplemental Table S3 for the row- and spikelet-related traits and Supplemental Table S4 for the age at start of growth.

treatment	ear rows	ear rows (% of control)	spikelets per row	spikelets per row (% of control)	spikelets per ear	spikelets per ear (% of Control)	Age at start of growth (DAE) & 95%CI	relative to control (days)
Control	13.1		51.4		670.4		49.4 (48.9; 49.9)	
V5-Drought	11.5	88.3 *	45.8	89.2 *	528.9	78.9 *	56.9 (55.6; 58.0)	7.5 *
V12-Drought	12.8	98.1	48.7	94.8 *	625.6	93.3 *	51.0 (50.5; 51.6)	1.6 *

Figure legends

Figure 1. Illustration of plant growth, development and drought treatments on the PHENOVISION platform. A. Illustration of plant growth and development over time in the PHENOVISION platform. For all treatments, images from left to right were taken at 15, 25, 35, 45, 55 and 65 days after plant emergence. Control plants (top row) were kept well-watered throughout their entire development. Drought-treated plants were kept well-watered until reaching the V5 or V12 developmental stage (middle or bottom row, respectively), after which the soil water content was lowered for the remainder of the experiment. The mean plant age at the start of the V5- and V12-Drought treatments is indicated by a red or yellow arrow, respectively. B. Visualization of the soil water content after daily watering throughout plant development for the Control, V5-Drought and V12-Drought treatments. Upon reaching V5 or V12 stage, target soil humidity was lowered from 2.4 g H₂O/g dry soil to 1.4 g H₂O/g dry soil. On average, V5-Drought plants did not receive water for 7 days (until their soil water content was sufficiently reduced), and V12-Drought plants did not receive water for 3 days on average.

Figure 2. Mean timing of organ growth and selected developmental stages for all treatments. Tall vertical lines indicate the mean plant age at the start of the drought treatments (16.3 DAE for V5-Drought, 39.6 DAE for V12-Drought). Open rectangles indicate the average growth timespan for the observed leaf ranks, with a vertical line inside the rectangle indicating the mean plant age at the emergence of the leaf from the whorl. Growth timespans were determined from three-piece-linear functions fitted to the length profiles of individual leaves. Filled rectangles indicate the elongation timespan for different subgroups of internodes (Int). Elongation timespans were determined from three-piece-linear functions fitted to destructive measurements from multiple plants throughout growth. Short vertical lines indicate the mean plant age at tassel emergence from the whorl (Tassel), ear husk leaf emergence (Ear), anthesis (Anthesis) and silk emergence (Silking). Sample numbers (n) for different leaf traits can be found in supplemental table S1. For the internode traits, n=48 for the Control treatment, n=49 for the V5-Drought treatment and n=22 for the V12-Drought treatment

(after V12-stage). Sample numbers for the reproductive developmental time points can be found in supplemental table S3.

Figure 3. Scatterplot of the observed values for mean LER (mm/day) on the x-axis and LED on the y-axis (days) for the different treatments and leaf ranks observed. The overall Pearson's correlation coefficient r between LED and mean LER is -0.96 at a p value $< 10^{-149}$. Exact sample numbers (n) for leaf growth curves can be found in supplemental table S1 for the traits LERmean and LED.

Figure 4. Growth curves for leaves, stems, stem fractions, and ears and ear development. A-C: Leaf elongation rates based on beta-sigmoid growth curves fitted to the length profiles of leaves 4, 6, 9, 12, 15 and 18 and their sum (gray) and smoothed sum (black), for the well-watered treatment (A), V5-Drought treatment (B), and V12-Drought treatment (C). Underlying leaf length data and fits are visualized in supplemental Fig. S4A-C. Exact sample numbers (n) for leaf growth curves can be found in supplemental table S1, under the trait FLL. D-F: Elongation rates for the well-watered treatment (D), V5-Drought treatment (E), and the V12-Drought treatment (F) based on beta sigmoid growth curves fitted to the length profiles of the stem fractions composed of internodes 1-8, 9-11, 12-14, 15-17 and 18-19 and the whole stem up to node 20 (black). An alternative approach to obtain the stem elongation rate is to take a smoothed sum of the elongation rates of the stem fractions (gray). Black vertical lines in B, C, E, F indicate the start of V5- or V12-Drought treatment. For the internode traits: $n=48$ for the Control treatment, $n=49$ for the V5-Drought treatment and $n=37$ for the V12-Drought treatment (22 V12-Drought samples taken after V12-stage, complemented with data from 15 Control-treated plants sampled before the V12-stage). G-H: Ear development over time, the number of ear rows (G) and spikelets per row (H) over time for the three treatments. Each circle represents a separate plant and lines are smoothing splines fitted to the measurements of all plants in a treatment. $n=119$ for Control, $n=116$ for V5-Drought, and $n=69$ for V12-Drought (of which 54 were V12-Drought treated plants after the V12-stage and 15 were Control treated plants before the V12-Stage). I: Ear elongation rate as derived from exponential growth functions fitted to ear length measurements over time for the three treatments. $n=97$ for Control, $n=101$ for V5-Drought, and $n=69$ for V12-Drought (of which 54 were V12-Drought treated plants after the V12-stage and 15 were Control treated plants before the V12-Stage). Yellow vertical lines in G, H, I indicate the start of the V12-Drought treatment. J: The development of the number of spikelets per ear compared to the ear elongation rate. Each circle represents a separate plant and lines are smoothing splines fitted to the measurements of all plants in a treatment. $n=90$ for Control, $n=90$ for V5-Drought and $n=63$ for V12-Drought (of which 54 were V12-Drought treated plants after the V12-stage and 9 were Control treated plants before the V12-Stage). Raw length data for A-F and I are shown in supplemental Fig. S4.

Figure 5. Performance of the image-based biomass estimation models. A: Performance of the image-based fresh weight prediction model. B: Performance of the image-based dry weight prediction model. Predictions were made using leave-one-out-cross-validation: each prediction is made using a model trained on all other data points. Destructively measured ground-truth biomass measurements are on the x-axes; image-based predictions using leave-one-out cross-validation are on the y-axes. $n=236$ (69 Control, 63 V5-Drought, 61 V5-Mild, 23 V12-Drought, 20 V12-Mild) for both fresh and dry weight. R^2 and Mean Absolute Percentage Error (MAPE) values are displayed in the top left.

Figure 6. Plant biomass and biomass accumulation rates over time. A: Estimated aboveground plant fresh weight over time, B: Estimated aboveground plant dry weight over time, C: Estimated plant fresh weight accumulation rate over time, D: Estimated plant dry weight accumulation rate over time. Thin lines connect daily estimates of weight of individual plants in A and B, while circles indicate individual estimates of accumulation rates in C and D. Thick lines trace the daily mean values

per treatment. Vertical dashed lines indicate the mean time of drought treatment initiation (V5-Drought: 16.3 DAE, V12-Drought: 39.6 DAE), and the colored rectangles directly above the x-axis indicate the periods in which there is a significant difference (Mann-Whitney U test with Holm-Bonferroni multiple testing correction, $p < 0.05$) between the respective drought treatment and the control treatment. Detachment and loss of lower leaves caused a loss of biomass and a perceived reduction and inaccurate estimation in biomass accumulation rate for all treatments during the period 40 DAE - 45 DAE. The total number of observations for both FW and DW is 24982 (9137 Control, 10778 V5-Drought and 5067 V12-Drought) for 897 unique observed plants (384 Control, 376 V5-Drought, 137 V12-Drought). The details regarding the number of observations for each day can be found for FW in supplemental table S8, DW in supplemental table S9, FW accumulation rate in supplemental table S6 and DW accumulation rate in supplemental table S7.

Figure 7. Normalized organ growth rates over time. The cumulative elongation rate of the leaves, the stem elongation rate and the ear elongation rate are all scaled relative to the peak value in the control treatment. Cumulative leaf growth rate is represented by a cubic smoothing spline fitted to the sum of the growth rates of the individual measured leaves. Stem growth rate is calculated as the first derivative of a beta-sigmoid growth function fitted to stem length measurements. Ear growth rate is calculated as the first derivative of an exponential growth function fitted to ear length measurement. Vertical dashed lines indicate the start of the drought treatments (V5-Drought: 16.3 DAE, V12-Drought: 39.6 DAE). This figure is supplemented with biomass accumulation data in supplemental Fig. S9A and treatments are combined in a single graph for comparing treatment effects in supplemental Fig. S9B.

Literature cited

- Anami SE, Mgtutu AJ, Taracha C, Coussens G, Karimi M, Hilson P, Van Lijsebettens M, Machuka J** (2010) Somatic embryogenesis and plant regeneration of tropical maize genotypes. *Plant Cell Tiss Organ Cult* **102**: 285–295
- Asaari MSM, Mertens S, Dhondt S, Inzé D, Wuyts N, Scheunders P** (2019) Analysis of hyperspectral images for detection of drought stress and recovery in maize plants in a high-throughput phenotyping platform. *Comput Electron Agric* **162**: 749–758
- Avramova V, Abdelgawad H, Vasileva I, Petrova AS, Holec A, Mariën J, Asard H, Beemster GTS** (2017) High Antioxidant Activity Facilitates Maintenance of Cell Division in Leaves of Drought Tolerant Maize Hybrids. *Front Plant Sci* **8**: 84
- Avramova V, Abdelgawad H, Zhang Z, Fotschki B, Casadevall R, Vergauwen L, Knapen D, Taleisnik E, Guisez Y, Asard H, et al** (2015) Drought Induces Distinct Growth Response, Protection and Recovery Mechanisms in the Maize Leaf Growth Zone. *Plant Physiol* **169**: 1382–1396
- Bechtold U, Penfold CA, Jenkins DJ, Legaie R, Moore JD, Lawson T, Matthews JSA, Violet-Chabrand SRM, Baxter L, Subramaniam S, et al** (2016) Time-Series Transcriptomics Reveals That AGAMOUS-LIKE22 Affects Primary Metabolism and Developmental Processes in Drought-Stressed Arabidopsis. *Plant Cell* **28**: 345–366
- Boyer JS** (1982) Plant Productivity and Environment. *Science* **218**: 443–448
- Boyer JS, Westgate ME** (2004) Grain yields with limited water. *J Exp Bot* **55**: 2385–2394

- Brichet N, Fournier C, Turc O, Strauss O, Artzet S, Pradal C, Welcker C, Tardieu F, Cabrera-Bosquet L** (2017) A robot-assisted imaging pipeline for tracking the growths of maize ear and silks in a high-throughput phenotyping platform. *Plant Methods* **13**: 96
- Chang T-G, Zhu X-G** (2017) Source–sink interaction: a century old concept under the light of modern molecular systems biology. *J Exp Bot* **68**: 4417–4431
- Claeys H, Inzé D** (2013) The Agony of Choice: How Plants Balance Growth and Survival under Water-Limiting Conditions. *Plant Physiol* **162**: 1768–1779
- Coussens G, Aesaert S, Verelst W, Demeulenaere M, De Buck S, Njuguna E, Inzé D, Van Lijsebettens M** (2012) Brachypodium distachyon promoters as efficient building blocks for transgenic research in maize. *J Exp Bot* **63**: 4263–4273
- Cramer GR, Van Sluyter SC, Hopper DW, Pascovici D, Keighley T, Haynes PA** (2013) Proteomic analysis indicates massive changes in metabolism prior to the inhibition of growth and photosynthesis of grapevine (*Vitis vinifera* L.) in response to water deficit. *BMC Plant Biol* **13**: 49
- Dhondt S, Wuyts N, Inzé D** (2013) Cell to whole-plant phenotyping: the best is yet to come. *Trends Plant Sci* **18**: 428–439
- Dias MC, Brüggemann W** (2010) Limitations of photosynthesis in *Phaseolus vulgaris* under drought stress: gas exchange, chlorophyll fluorescence and Calvin cycle enzymes. *Photosynthetica* **48**: 96–102
- Edmeades GO, Bolaños J, Hernández M, Bello S** (1993) Causes for Silk Delay in a Lowland Tropical Maize Population. *Crop Sci* **33**: 1029–1035
- Fahlgren N, Gehan MA, Baxter I** (2015) Lights, camera, action: high-throughput plant phenotyping is ready for a close-up. *Curr Opin Plant Biol* **24**: 93–99
- Frame BR, McMurray JM, Fonger TM, Main ML, Taylor KW, Torney FJ, Paz MM, Wang K** (2006) Improved Agrobacterium-mediated transformation of three maize inbred lines using MS salts. *Plant Cell Rep* **25**: 1024–1034
- Freeling M, Walbot V, eds** (1994) *The Maize Handbook*. Springer New York, New York, NY
- Gururani MA, Venkatesh J, Tran LSP** (2015) Regulation of Photosynthesis during Abiotic Stress-Induced Photoinhibition. *Mol Plant* **8**: 1304–1320
- Hall AJ, Vilella F, Trapani N, Chimenti C** (1982) The effects of water stress and genotype on the dynamics of pollen-shedding and silking in maize. *Field Crops Res* **5**: 349–363
- Hari V, Rakovec O, Markonis Y, Hanel M, Kumar R** (2020) Increased future occurrences of the exceptional 2018–2019 Central European drought under global warming. *Sci Rep* **10**: 12207
- Harrison MT, Tardieu F, Dong Z, Messina CD, Hammer GL** (2014) Characterizing drought stress and trait influence on maize yield under current and future conditions. *Glob Change Biol* **20**: 867–878
- Hay RKM** (1995) Harvest index: a review of its use in plant breeding and crop physiology. *Ann Appl Biol* **126**: 197–216
- Hisanaga T, Kawade K, Tsukaya H** (2015) Compensation: a key to clarifying the organ-level regulation of lateral organ size in plants. *J Exp Bot* **66**: 1055–1063

- Holm S** (1979) A Simple Sequentially Rejective Multiple Test Procedure. *Scand Stat Theory Appl* **6**: 65–70
- Jedrowski C, Bayramov S, Brüggemann W** (2014) Comparative analysis of drought stress effects on photosynthesis of Eurasian and North African genotypes of wild barley. *Photosynthetica* **52**: 564–573
- Kenny GJ, Harrison PA** (1992) Thermal and moisture limits of grain maize in Europe: model testing and sensitivity to climate change. *Clim Res* **2**: 113–129
- Lawlor DW** (2013) Genetic engineering to improve plant performance under drought: physiological evaluation of achievements, limitations, and possibilities. *J Exp Bot* **64**: 83–108
- Lehner F, Coats S, Stocker TF, Pendergrass AG, Sanderson BM, Raible CC, Smerdon JE** (2017) Projected drought risk in 1.5°C and 2°C warmer climates: Drought in 1.5°C and 2°C Warmer Climates. *Geophys Res Lett* **44**: 7419–7428
- Liu S, Martre P, Buis S, Abichou M, Andrieu B, Baret F** (2019) Estimation of Plant and Canopy Architectural Traits Using the Digital Plant Phenotyping Platform. *Plant Physiol* **181**: 881–890
- Lobell DB, Roberts MJ, Schlenker W, Braun N, Little BB, Rejesus RM, Hammer GL** (2014) Greater Sensitivity to Drought Accompanies Maize Yield Increase in the U.S. Midwest. *Science* **344**: 516–519
- Nelissen H, Gonzalez N, Inzé D** (2016) Leaf growth in dicots and monocots: so different yet so alike. *Curr Opin Plant Biol* **33**: 72–76
- Nelissen H, Rymen B, Jikumar Y, Demuynck K, Van Lijsebettens M, Kamiya Y, Inzé D, Beemster GTS** (2012) A Local Maximum in Gibberellin Levels Regulates Maize Leaf Growth by Spatial Control of Cell Division. *Curr Biol* **22**: 1183–1187
- Nelissen H, Sun X-H, Rymen B, Jikumar Y, Kojima M, Takebayashi Y, Abbeloos R, Demuynck K, Storme V, Vuylsteke M, et al** (2018) The reduction in maize leaf growth under mild drought affects the transition between cell division and cell expansion and cannot be restored by elevated gibberellic acid levels. *Plant Biotechnol J* **16**: 615–627
- Passioura JB, Fry SC** (1992) Turgor and Cell Expansion: Beyond the Lockhart Equation. *Funct Plant Biol* **19**: 565–576
- Pastenes C, Pimentel P, Lillo J** (2005) Leaf movements and photoinhibition in relation to water stress in field-grown beans. *J Exp Bot* **56**: 425–433
- R Core Team** (2020) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria
- Ritchie SW, Hanway JJ, Benson GO** (1993) How a corn plant develops. Iowa State University of Science and Technology, Cooperative Extension Service, Ames, Iowa
- Rodrigues J, Inzé D, Nelissen H, Saibo NJM** (2019) Source–Sink Regulation in Crops under Water Deficit. *Trends Plant Sci* **24**: 652–663
- Saini HS, Westgate ME** (1999) Reproductive Development in Grain Crops during Drought. *Adv Agron* **68**: 59–96

- Siemer EG, Leng ER, Bonnett OT** (1969) Timing and Correlation of Major Developmental Events in Maize, *Zea mays* L. *Agron J* **61**: 14–17
- Struik PC, Doorgeest M, Boonman JG** (1986) Environmental effects on flowering characteristics and kernel set of maize (*Zea mays* L.). *Neth J Agric Sci* **34**: 469–484
- Sun X, Cahill J, Van Hautegeem T, Feys K, Whipple C, Novák O, Delbare S, Versteede C, Demuynck K, De Block J, et al** (2017) Altered expression of maize *PLASTOCHRON1* enhances biomass and seed yield by extending cell division duration. *Nat Commun* **8**: 14752
- Toreti A, Belward A, Perez-Dominguez I, Naumann G, Luterbacher J, Cronie O, Seguini L, Manfron G, Lopez-Lozano R, Baruth B, et al** (2019) The Exceptional 2018 European Water Seesaw Calls for Action on Adaptation. *Earth's Future* **7**: 652–663
- Tovar JC, Hoyer JS, Lin A, Tielking A, Callen ST, Castillo SE, Miller M, Tessman M, Fahlgren N, Carrington JC, et al** (2018) Raspberry Pi-powered imaging for plant phenotyping. *Applications in Plant Sciences* **6**: 1–12
- Vos J, Evers JB, Buck-Sorlin GH, Andrieu B, Chelle M, de Visser PHB** (2010) Functional–structural plant modelling: a new versatile tool in crop science. *J Exp Bot* **61**: 2101–2115
- Wallace DH, Baudoin JP, Beaver J, Coyne DP, Halseth DE, Masaya PN, Munger HM, Myers JR, Silbernagel M, Yourstone KS, et al** (1993) Improving efficiency of breeding for higher crop yield. *Theoret Appl Genetics* **86**: 27–40
- Wallace JS** (2000) Increasing agricultural water use efficiency to meet future food production. *Agric Ecosyst Environ* **82**: 105–119
- White AC, Rogers A, Rees M, Osborne CP** (2016) How can we make plants grow faster? A source–sink perspective on growth rate. *J Exp Bot* **67**: 31–45
- Wickham H** (2016) *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York

Parsed Citations

Anami SE, Mgutu AJ, Taracha C, Coussens G, Karimi M, Hilson P, Van Lijsebettens M, Machuka J (2010) Somatic embryogenesis and plant regeneration of tropical maize genotypes. *Plant Cell Tiss Organ Cult* 102: 285–295

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Asaari MSM, Mertens S, Dhondt S, Inzé D, Wuyts N, Scheunders P (2019) Analysis of hyperspectral images for detection of drought stress and recovery in maize plants in a high-throughput phenotyping platform. *Comput Electron Agric* 162: 749–758

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Avramova V, AbdElgawad H, Vasileva I, Petrova AS, Holec A, Mariën J, Asard H, Beemster GTS (2017) High Antioxidant Activity Facilitates Maintenance of Cell Division in Leaves of Drought Tolerant Maize Hybrids. *Front Plant Sci* 8: 84

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Avramova V, AbdElgawad H, Zhang Z, Fotschki B, Casadevall R, Vergauwen L, Knapen D, Taleisnik E, Guisez Y, Asard H, et al (2015) Drought Induces Distinct Growth Response, Protection and Recovery Mechanisms in the Maize Leaf Growth Zone. *Plant Physiol* 169: 1382–1396

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Bechtold U, Penfold CA, Jenkins DJ, Legaie R, Moore JD, Lawson T, Matthews JSA, Vialet-Chabrand SRM, Baxter L, Subramaniam S, et al (2016) Time-Series Transcriptomics Reveals That AGAMOUS-LIKE22 Affects Primary Metabolism and Developmental Processes in Drought-Stressed Arabidopsis. *Plant Cell* 28: 345–366

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Boyer JS (1982) Plant Productivity and Environment. *Science* 218: 443–448

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Boyer JS, Westgate ME (2004) Grain yields with limited water. *J Exp Bot* 55: 2385–2394

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Brichet N, Fournier C, Turc O, Strauss O, Artzet S, Pradal C, Welcker C, Tardieu F, Cabrera-Bosquet L (2017) A robot-assisted imaging pipeline for tracking the growths of maize ear and silks in a high-throughput phenotyping platform. *Plant Methods* 13: 96

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Chang T-G, Zhu X-G (2017) Source–sink interaction: a century old concept under the light of modern molecular systems biology. *J Exp Bot* 68: 4417–4431

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Claeys H, Inzé D (2013) The Agony of Choice: How Plants Balance Growth and Survival under Water-Limiting Conditions. *Plant Physiol* 162: 1768–1779

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Coussens G, Aesaert S, Verelst W, Demeulenaere M, De Buck S, Njuguna E, Inzé D, Van Lijsebettens M (2012) Brachypodium distachyon promoters as efficient building blocks for transgenic research in maize. *J Exp Bot* 63: 4263–4273

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Cramer GR, Van Sluyter SC, Hopper DW, Pascovici D, Keighley T, Haynes PA (2013) Proteomic analysis indicates massive changes in metabolism prior to the inhibition of growth and photosynthesis of grapevine (*Vitis vinifera* L.) in response to water deficit. *BMC Plant Biol* 13: 49

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Dhondt S, Wuyts N, Inzé D (2013) Cell to whole-plant phenotyping: the best is yet to come. *Trends Plant Sci* 18: 428–439

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Dias MC, Brüggemann W (2010) Limitations of photosynthesis in *Phaseolus vulgaris* under drought stress: gas exchange, chlorophyll fluorescence and Calvin cycle enzymes. *Photosynthetica* 48: 96–102

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Edmeades GO, Bolaños J, Hernández M, Bello S (1993) Causes for Silk Delay in a Lowland Tropical Maize Population. *Crop Sci* 33: 1029–1035

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Fahlgren N, Gehan MA, Baxter I (2015) Lights, camera, action: high-throughput plant phenotyping is ready for a close-up. *Curr Opin Plant Biol* 24: 93–99

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Frame BR, McMurray JM, Fonger TM, Main ML, Taylor KW, Torney FJ, Paz MM, Wang K (2006) Improved Agrobacterium-mediated transformation of three maize inbred lines using MS salts. *Plant Cell Rep* 25: 1024–1034

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Freeling M, Walbot V, eds (1994) *The Maize Handbook*. Springer New York, New York, NY

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Gururani MA, Venkatesh J, Tran LSP (2015) Regulation of Photosynthesis during Abiotic Stress-Induced Photoinhibition. *Mol Plant* 8: 1304–1320

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Hall AJ, Vilella F, Trapani N, Chimenti C (1982) The effects of water stress and genotype on the dynamics of pollen-shedding and silking in maize. *Field Crops Res* 5: 349–363

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Hari V, Rakovec O, Markonis Y, Hanel M, Kumar R (2020) Increased future occurrences of the exceptional 2018–2019 Central European drought under global warming. *Sci Rep* 10: 12207

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Harrison MT, Tardieu F, Dong Z, Messina CD, Hammer GL (2014) Characterizing drought stress and trait influence on maize yield under current and future conditions. *Glob Change Biol* 20: 867–878

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Hay RKM (1995) Harvest index: a review of its use in plant breeding and crop physiology. *Ann Appl Biol* 126: 197–216

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Hisanaga T, Kawade K, Tsukaya H (2015) Compensation: a key to clarifying the organ-level regulation of lateral organ size in plants. *J Exp Bot* 66: 1055–1063

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Holm S (1979) A Simple Sequentially Rejective Multiple Test Procedure. *Scand Stat Theory Appl* 6: 65–70

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Jedrowski C, Bayramov S, Brüggemann W (2014) Comparative analysis of drought stress effects on photosynthesis of Eurasian and North African genotypes of wild barley. *Photosynthetica* 52: 564–573

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Kenny GJ, Harrison PA (1992) Thermal and moisture limits of grain maize in Europe: model testing and sensitivity to climate change. *Clim Res* 2: 113–129

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Lawlor DW (2013) Genetic engineering to improve plant performance under drought: physiological evaluation of achievements, limitations, and possibilities. *J Exp Bot* 64: 83–108

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Lehner F, Coats S, Stocker TF, Pendergrass AG, Sanderson BM, Raible CC, Smerdon JE (2017) Projected drought risk in 1.5°C and 2°C warmer climates: Drought in 1.5°C and 2°C Warmer Climates. *Geophys Res Lett* 44: 7419–7428

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Liu S, Martre P, Buis S, Abichou M, Andrieu B, Baret F (2019) Estimation of Plant and Canopy Architectural Traits Using the Digital Plant Phenotyping Platform. *Plant Physiol* 181: 881–890

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Lobell DB, Roberts MJ, Schlenker W, Braun N, Little BB, Rejesus RM, Hammer GL (2014) Greater Sensitivity to Drought Accompanies Maize Yield Increase in the U.S. Midwest. *Science* 344: 516–519

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Nelissen H, Gonzalez N, Inzé D (2016) Leaf growth in dicots and monocots: so different yet so alike. *Curr Opin Plant Biol* 33: 72–76

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Nelissen H, Rymen B, Jikumaru Y, Demuyne K, Van Lijsebettens M, Kamiya Y, Inzé D, Beemster GTS (2012) A Local Maximum in Gibberellin Levels Regulates Maize Leaf Growth by Spatial Control of Cell Division. *Curr Biol* 22: 1183–1187

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Nelissen H, Sun X-H, Rymen B, Jikumaru Y, Kojima M, Takebayashi Y, Abbeloos R, Demuyne K, Storme V, Vuylsteke M, et al (2018) The reduction in maize leaf growth under mild drought affects the transition between cell division and cell expansion and cannot be restored by elevated gibberellic acid levels. *Plant Biotechnol J* 16: 615–627

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Passioura JB, Fry SC (1992) Turgor and Cell Expansion: Beyond the Lockhart Equation. *Funct Plant Biol* 19: 565–576

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Pastenes C, Pimentel P, Lillo J (2005) Leaf movements and photoinhibition in relation to water stress in field-grown beans. *J Exp Bot* 56: 425–433

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

R Core Team (2020) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Ritchie SW, Hanway JJ, Benson GO (1993) How a corn plant develops. Iowa State University of Science and Technology, Cooperative

Extension Service, Ames, Iowa

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Rodrigues J, Inzé D, Nelissen H, Saibo NJM (2019) Source–Sink Regulation in Crops under Water Deficit. Trends Plant Sci 24: 652–663

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Saini HS, Westgate ME (1999) Reproductive Development in Grain Crops during Drought. Adv Agron 68: 59–96

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Siemer EG, Leng ER, Bonnett OT (1969) Timing and Correlation of Major Developmental Events in Maize, *Zea mays* L. Agron J 61: 14–17

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Struik PC, Doorgeest M, Boonman JG (1986) Environmental effects on flowering characteristics and kernel set of maize (*Zea mays* L.). Neth J Agric Sci 34: 469–484

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Sun X, Cahill J, Van Hautegeem T, Feys K, Whipple C, Novák O, Delbare S, Verstelee C, Demuyne K, De Block J, et al (2017) Altered expression of maize PLASTOCHRON1 enhances biomass and seed yield by extending cell division duration. Nat Commun 8: 14752

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Toreti A, Belward A, Perez-Dominguez I, Naumann G, Luterbacher J, Cronie O, Seguini L, Manfron G, Lopez-Lozano R, Baruth B, et al (2019) The Exceptional 2018 European Water Seesaw Calls for Action on Adaptation. Earth's Future 7: 652–663

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Tovar JC, Hoyer JS, Lin A, Tielking A, Callen ST, Castillo SE, Miller M, Tessman M, Fahlgren N, Carrington JC, et al (2018) Raspberry Pi–powered imaging for plant phenotyping. Applications in Plant Sciences 6: 1–12

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Vos J, Evers JB, Buck-Sorlin GH, Andrieu B, Chelle M, de Visser PHB (2010) Functional–structural plant modelling: a new versatile tool in crop science. J Exp Bot 61: 2101–2115

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Wallace DH, Baudoin JP, Beaver J, Coyne DP, Halseth DE, Masaya PN, Munger HM, Myers JR, Silbernagel M, Yourstone KS, et al (1993) Improving efficiency of breeding for higher crop yield. Theoret Appl Genetics 86: 27–40

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Wallace JS (2000) Increasing agricultural water use efficiency to meet future food production. Agric Ecosyst Environ 82: 105–119

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

White AC, Rogers A, Rees M, Osborne CP (2016) How can we make plants grow faster? A source–sink perspective on growth rate. J Exp Bot 67: 31–45

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Wickham H (2016) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)