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Environmental Systems Science

Genetic determinants of leaf growth response to environment in wheat

Master Thesis

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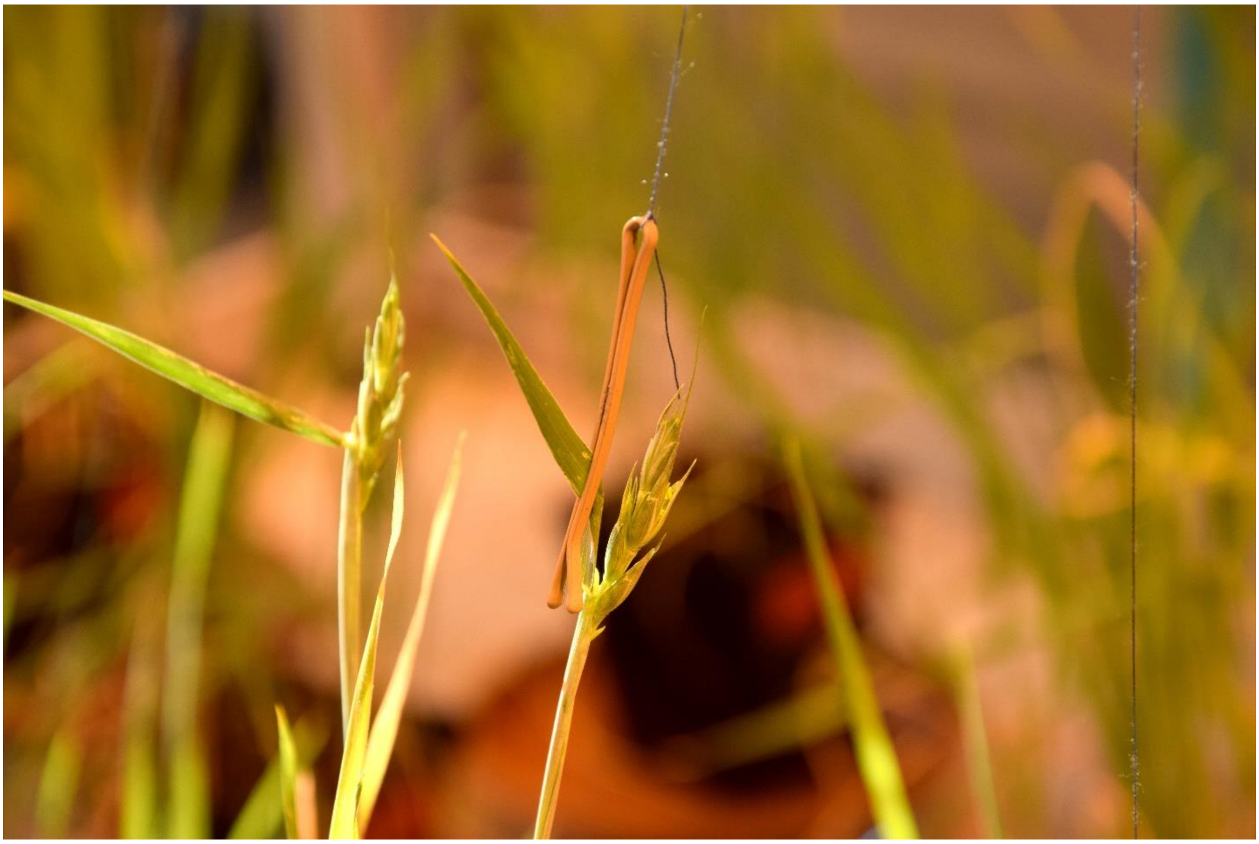

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Abstract

An increase in crop water stress is expected in many regions over coming decades. Therefore, there is a need for drought tolerant and high yielding wheat varieties to ensure global food security. Breeding on drought tolerance has proven to be difficult as there is no fast, automated and reproducible phenotyping method linked to yield under water stress. In this study, we present a method measuring leaf elongation rate (LER) on a high temporal resolution. 320 wheat varieties with three replicates were grown for one week in a greenhouse and were exposed to increasing water stress. LER was measured along with temperature, air humidity, light and gravimetric water content (GWC) of the substrate. Genotype specific response curves to environmental variables were used to model LER. The resulting model was able to predict LER of an unseen data set (R^2 = 0.40). A genome wide association study (GWAS) resulted in some interesting candidate genes for genotype specific drought response which might be further examined. The entire phenotyping process was cheap and could easily be adapted by breeders. It led to a rough characterization of drought tolerance within three weeks. This opens the way for selection on drought tolerance at an early breeding stage.

Keywords: water stress, drought, LER, plant growth, wheat, phenotyping, GWAS

Contents

Abbreviations

G×E	Genotype × environment	R^2	Coefficient of determination
GWAS	Genome-wide association study	sd	Standard deviation
GWC	Gravimetric water content	SW	Spring wheat
LED	Leaf elongation duration	SWC	Soil water content
LER	Leaf elongation rate per unit time	VPD	Vapour pressure deficit
MEU	Monocot envirotyping unit	WUE	Water use efficiency
QTL	Quantitative trait loci	WW	Winter wheat

1. Introduction

The world population is projected to reach 9.8 billion by 2050 (UNO, 2017). Accordingly, an increased demand for agricultural products, especially food, is a consequence. At the same time, production systems are increasingly under pressure due to climate change. The IPCC (2013) projects a decrease in mean precipitation in many mid-latitude and subtropical dry regions. Worldwide, the contrast in precipitation between wet and dry regions as well as between wet and dry seasons is expected to increase. There can be regional deviations from the global trend. In Switzerland, the prediction models expect an increase in winter precipitation, heavy precipitation events and droughts (Scherrer et al., 2016a; Scherrer et al., 2016b)et al. In particular the summer will become drier with a 21–28% decrease of summer mean precipitation (Fischer et al., 2012).

Change in water availability could have a large impact on the productivity of our current varieties. Already today, plant growth is limited by water availability on about 40% of the earths vegetated surface (Nemani et al., 2003). An increase of at least 146% of human appropriated freshwater would be necessary to close the water related yield gap on current agriculturally used areas (Davis et al., 2017). This large amount of additional freshwater resource is simply not available in most countries. Additionally, irrigation is often uneconomic for low value crops like wheat. A more efficient use of the available water resources is unavoidable to achieve the goals of sustainable intensification. The choice of crop as well as a breeding improvement of the varieties are key factors to become more water efficient and more resilient against water stress.

Traditionally, plant breeding was focusing on improving yield, quality and resistance against pest and diseases mainly in favourable environments. Drought tolerance and water use efficiency (WUE) are relatively new breeding goals. Nevertheless, empirical observations show a fast growth in wheat yield potential in marginal environments. Between 1980 and 2000 the rate of yield gain in drought environments was twice that of favourable environments (Lantican et al., 2003). During the immediate post-Green Revolution period, the high-yielding varieties bred for favourable environments were used to develop new varieties also for marginal environments. The success of the marginal environment breeding was mainly based on the progress in the elite breeding for favourable environments (spill-over effect). Since the 1990s, the use of marginal environment germplasm has gained importance in breeding programmes (Lantican et al., 2003).

Despite large breeding efforts and fast yield increase, the yield potential of drought prone wheat varieties is still low compared to varieties bred for sufficient water availability. Several factors hamper a fast and efficient breeding for drought tolerant wheat so far: (i) Drought tolerance is a complex trait which is controlled by multiple genes with minor effect on the trait (Tuberosa, 2012). Adding or knocking out a single quantitative trait locus (QTLs) will probably have a small effect on the phenotype as it might be buffered by other components in the complex growth regulatory network (Vanhaeren et al., 2016). (ii) Bread wheat (*Triticum aestivum L.*) is an allohexaploid species with a genome size close to 16 giga base pairs (Zimin et al., 2017). It is one of the largest and most complex genomes of all crop species. Genetic dissection of certain traits becomes more difficult with genome size and genome complexity. (iii) Droughts occur irregularly and vary in length and severity. Regular selection for drought tolerance in an uncontrolled environment is impossible. (iv) The genotype × environment (G×E) interaction in drought experiments usually leads to low heritability. (v) There is no standardized and easy-to-use method to phenotype the plant's response to water stress (Mwadzingeni et al., 2016). A simple and efficient phenotyping is a prerequisite for breeding on drought tolerance.

The complexity of the drought tolerance inheritance and of the wheat genome cannot be simplified. Only the last two points can be addressed by a suitable experimental design and a simple and reproducible method, which measures a drought related trait in a controlled environment. The data needs to be combined with precise environmental measurements to split the overall effect into effects of the genotype, environment and the G×E interaction. The perfect method should be fast, automated, able to predict yields under water stress accurately and it has to be reproducible. In literature we found a wide variety of methods targeting different traits related to drought tolerance and drought avoidance like early vigour, root architecture, carbon isotope discrimination, stomatal conductance, canopy temperature, abscisic acid concentration, osmotic adjustment, stay-green mechanisms and remobilization of water soluble carbohydrates (well reviewed by Tuberosa (2012)). Measuring yield under drought stress would be the most direct way but require an entire growing season and a large experimental plot which is not available at an early selection stage (Shafeeq and Zafar, 2006). Similar problems are faced by remote sensing approaches, measuring spectral reflectance, canopy cover or canopy temperature. Those methods come at a low cost but correlation to yield might be spurious rather than causal and therefore change over time. The measurements are also largely affected by the environmental inference and therefore lack reproducibility in different environments (Yu et al., 2017). Precise physiological methods are directly connected to yield but are labour intensive and difficult to automate which again is a clear disadvantage in an early selection cycle. Therefore, we are looking for a physiological trait, directly related to yield but easy to measure with a high throughput approach. In this study we present a fast method to assess the plant response to water stress by measuring the leaf elongation rate (LER) with a low-cost phenotyping platform.

Growth rates are generally expected to be a good indicator for the welfare of a plant (Friedli, 2015). Cramer et al. (2011) even defines abiotic stress through its negative effect on plant growth. Therefore, it is a good trait to measure the stress tolerance of a certain variety. Monocot leaves are mainly growing linearly in one direction which makes LER a good candidate to measure response to stress in vivo. Compared to stem elongation or ear growth, LER measurements can start shortly after emergence and growth response to environmental conditions are detectable within minutes which makes it a fast phenotyping method Lacube et al. (2017). Techniques for an automated LER tracking are already developed and have been tested in many different studies (Friedli, 2015; Nagelmüller et al., 2016; Sadok et al., 2007b). Fast leaf area expansion has been shown to be positively correlated with grain yield (van den Boogaard et al., 1996). The link between LER and yield might be based on two mechanisms (see also chapter 2.1.8 and 2.2.5): (i) high intrinsic growth rate leads to a rapid leaf area development during early crop stages which has a positive effect on yield especially in water limited Mediterranean climate (Bultynck et al., 2004).

(ii) growth maintenance under mild water deficit leads to higher biomass accumulation which finally translate into a higher yield (Chenu et al., 2008). The reproducibility of LER response to water deficit between glasshouse and field is low due to the large overriding effect of temperature and vapour pressure deficit (VPD). A rigorous control of micrometeorological conditions would not be practicable and thus prevent widespread use of the method. However, Sadok et al. (2007b) were able to make LER temperature independent by expressing it per unit thermal time. The effect of other environmental variables can be estimated and considered in the same way, making LER comparable and reproducible across different days, experiments, environmental conditions and locations (Lacube et al., 2017). Reproducible LER responses can be used to detect stable QTLs related to growth or growth maintenance under water stress (Reymond et al., 2003). Many studies characterized LER response in maize but to our knowledge an application on drought tolerance detection in wheat has not been tested so far.

The aim of this study was to test LER measurements as a fast screening method to find wheat varieties which might perform better under a certain drought scenario. To this end, we measured the LER of 320 wheat varieties on a new phenotyping platform and under changing environmental conditions. The response curves of LER were quantified in an environmental modelling process. Important genotype specific parameters of the model were compared with growth response curves of other studies using the same wheat varieties. Finally genotype specific model parameters were used in genome-wide association study (GWAS) in order to analyse the molecular genetic mechanism that govern LER under water stress conditions.

Based on those aims the study addresses the following research questions: (i) Is it possible to make accurate LER measurements with high temporal resolution on a low-cost phenotyping platform. (ii) Can LER be modelled with high precision based on genotype specific response curves to environmental variables. (iii) What are the differences between varieties in their response to drought stress. (iv) Which genes are associated with the parameter values of our LER model.

2. Theory

2.1 Plant growth

2.1.1 Importance of growth

Growth is a key factor in the development of a plant influencing its survival, fitness and competitiveness. In contrast to other organisms, plants are sessile and can only access new resources through growth. Leaf growth is essential to increase the light interception capacity whereas root growth increases the rhizosphere and gives access to additional water and nutrient resources. Growth is regulated to prevent uneconomical use of limiting resources. For example, leaf growth in a water limited environment can lead to higher water transpiration rate and thereby endangers the survival of the plant. Therefore, plant growth has to react to changing environmental conditions especially for optimize resource use efficiency (Walter et al. 2009).

2.1.2 Cellular growth

Growth can be defined by the irreversible addition and expansion of cells (Mencuccini et al., 2017). The definition already mentions the two fundamental and distinct processes involved in plant growth. All cells for primary and secondary growth are produced by cell division at the shoot and root apical meristems (Taiz and Zeiger, 2003). The cell production rate depends on the meristematic cell division rate and the number of meristematic cells. Cell division alone does not lead to an expansion of the plant substance but only to an increase in number of cells per unit area (Ben-Haj-Salah and Tardieu, 1995). Division only affects plant growth in combination with the second growth process, cell expansion. The size of a plant cell is limited by the cell wall, a complex structural layer evolved to withstand the tensile force caused by the osmotic pressure of the protoplast. Cell expansion is only possible by increasing the plasticity of the cell wall. Plants actively regulate the plasticity with a number of processes and agents including expansins, xyloglucan hydrolases, xyloglucan endotransglucosylases, cellulases and hydroxyl radicals. All those processes are currently subject of intensive research (Bashline et al., 2014; Cosgrove, 2005). Growth is finally the product of cell production rate times the final cell volume (Kavanová et al., 2006).

2.1.3 Organ growth

In this experiment we focus on leaf growth. The development and growth of a wheat leaf follows a defined procedure and is comparable to the leaf development of all members of the Poaceae family (Fig. 1). Leaf primordiums (groups of cells that will develop into a leaf) are distichously formed on the flanks of the shoot apical meristem (Kellogg, 2015). Initially the entire primordium is meristematic tissue with cells dividing and expanding throughout. With increasing leaf size, the cell division is limited to the basis of the leaf while the distal cells differentiate into specialized cells. Newly created cells at the proliferative zone at the base of the leaf (intercalary meristem) continuously push older cells in distal direction (Ben-Haj-Salah and Tardieu, 1995). Ligule and sheath develop within the proliferative zone and split the zone into two parts (Sylvester and Smith, 2009). The lower part produces cells for the leaf sheath and upper part for the leaf blade. Both regions form a developmental gradient in distal direction starting with the cell division zone followed by a cell expansion zone and ending with mature leaf tissue. The region of division and expansion are partly overlapping in time and space (Ben-Haj-Salah and Tardieu, 1995). Together they are described as the leaf growth zone (Fig. 2).

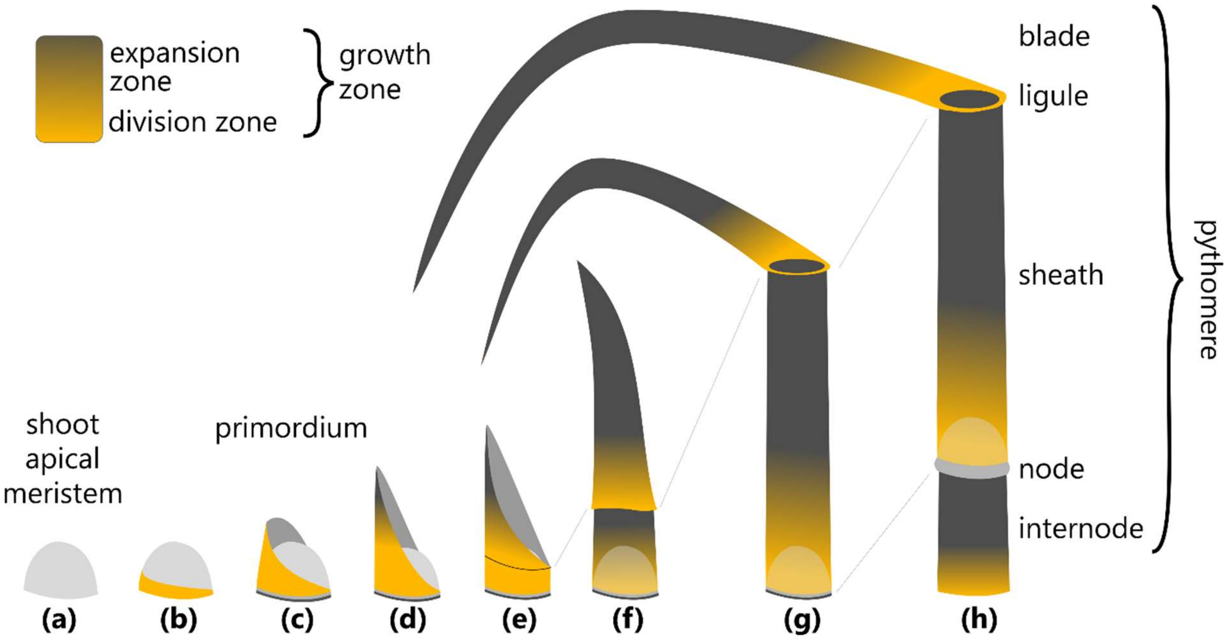


Fig. 1: Phytomere development in monocots. (a) Meristematic cells at the shoot apical meristem are the starting point of each organ initiation. (b) 200 founder cells are recruited to build new phytomere. (c) Cells multiply and built a primordium. Initiation of node and internode at the insertion area of the primordium. (d) Cell expansion and differentiation of distal cells. Cell division is restricted lower part of the growth zone, the so-called proliferation zone. (e) Proliferative zone is split by the developing ligule. (f) two developmental gradients along the leaf. (g) Growth of blade and sheath. (h) Additional growth zone at base of internode. Lift node, leaf and apical meristem to a higher level. Figure adapted from Scanlon et al. 2003 and expanded according to descriptions in Kellogg 2015 and Sylvester et al 2009.

The spatial distribution of tissue expansion rate in the leaf growth zone of the blade is well described for several monocot species including wheat (Beemster and Masle, 1996; Hu and Schmidhalter, 2008; Masle, 2000), maize (Tardieu et al., 2000), *Lolium perenne* (Kavanová et al., 2006; Kavanová et al., 2008), *Festuca arundinacea* (MacAdam et al., 1989), two *Aegilops* species (Bultynck et al., 2003) and several *Poa* species (Fiorani et al., 2000).

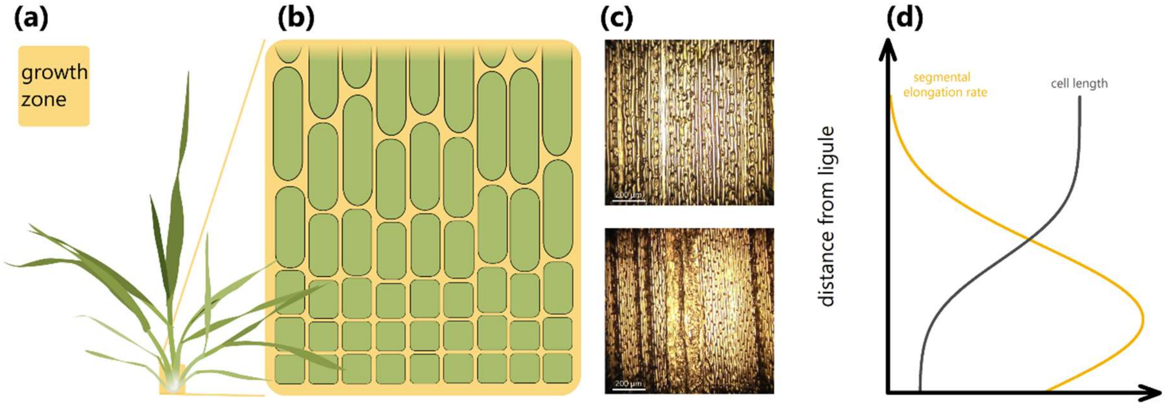


Fig. 2: Adaxial epidermis of leaf growth zone. (a) located at the base of the plant close to the ground and protected by leaf sheath of previous leaf. (b) cell size gradient along growth zone. (c) Microscope images of adaxial epidermis cells in the division zone and the elongation zone. (d) elongation rate has a maximum within the elongation zone and decreases in distal direction. According to data and figures in Muller et al. 2001 and Ben-Haj-Salah et al. 1995.

The size of the growth zone in wheat is in the range of 21 to 35 mm with the cell division only taking place in the first 3 to 7 mm (Beemster et al., 1996; Masle, 2000). 85 to 90% of the overall leaf elongation is generated in the elongation only zone where division no longer occurs (Beemster et al., 1996). However, those results were obtained by only considering epidermal cells. Ben-Haj-Salah and Tardieu (1995) showed in maize a three times longer division zone for mesophyll cells compared to epidermal cells. Several studies showed a positive correlation between size of the cell division zone and LER by comparing different species (Arredondo and Schnyder, 2003; Bultynck et al., 2003), different genotypes of one species (Baute et al., 2016) or different leaves within one plant (Beemster et al., 1996; Nelissen et al., 2012).

Each leaf is attached to the shoot by a node. Leaf, node, internode and axillary bud together build one phytomer which can be considered as the fundamental building block of plants (McMaster, 2005). Internodes emerge from cells on the stem just below the insertion of each primordia (Fournier and Andrieu, 2000). The whole apical cone elongates very slowly as a single entity as long as the plant is in the vegetative stage (Siemer et al., 1969). The plant changes to the reproductive stage when the shoot apex stops producing leaves and starts producing spikelets. The period of rapid stem elongation starts shortly after the initiation of the terminal spikelet (Kirby, 1985). Internodes form a growing zone comparable to those of the leaf blade and leaf sheath with a cell division and a cell expansion zone (Kende et al., 1998). The Internodes elongate in an ordered sequence starting with the lower internodes. Some internodes at the bottom of the plant are not elongating (Kirby et al., 1994). The internode growth leads to a raising of the nodes and the leaves in the canopy.

During the period of vegetative growth, mainly two separate meristematic regions contribute to the leaf growth namely the meristematic region at the base of the blade and the meristematic region at the base of the sheath. The timing of the sheath expansion is slightly delayed but both processes are temporally overlapping (Fournier and Andrieu, 1998). During the period of rapid stem elongation, leaf elongation is still ongoing until the growth stage of booting (McMaster, 2005). In this period, a change in distance between leaf tip and ground results from the cumulative elongation of at least three separate meristematic regions including the two leaf meristems and (multiple) internode meristems (Fig 1h).

Elongation rate of blade, sheath and internode over time follows a sigmoidal curve with lower growth rate at the start and end of elongation (Fig. 3) (Kirby, 1988; Kirby et al., 1994; Masle, 2000). In between there is a period of more or less linear growth rate (i.e. rate independent of leaf length). Final blade, sheath and internode length depend on its position within the plant. Generally, blade, sheath and internode length increase with leaf rank until a certain limit. Weightman et al. (1997) showed a constant increase of leaf blade length with leaf number except for the flag leaf which was shorter than the previous. The same was shown for the Internode by Kirby et al. (1994) and are also observed in Maize (Fournier and Andrieu, 2000). Sheath length in Maize was found to decrease already earlier from leaf number seven on (Andrieu et al., 2006)

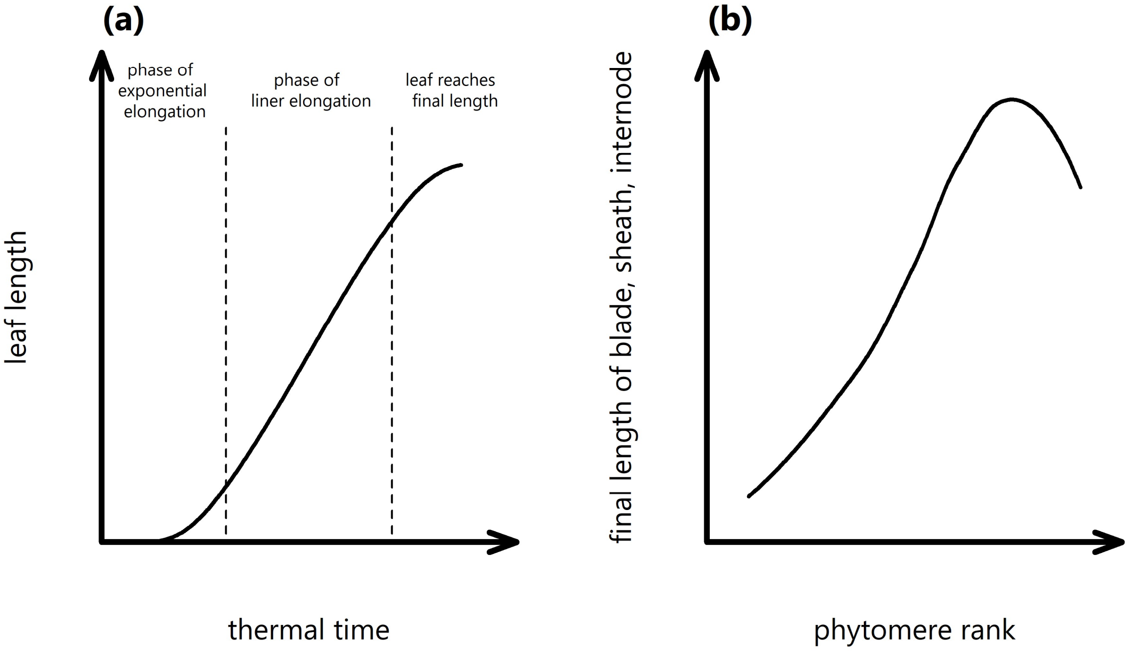


Fig. 3: Leaf length over time and phytomere. (a) After being initiated at the shoot apical meristem, leaf length increases exponential until elongation zone reaches its final length. Afterwards a relatively long period of linear elongation is observed. Finally, elongation rate slows down as the leaf reaches its final length. (b) Final length of blade, sheath and internode increases with phytomere rank until a certain limit. According to data and figures in Muller et al. 2001, Weightman et al 1997, Masle 2000, Fournier et al. 2000, Kirby et al. 1994 and Kirby et al. 1988.

2.1.4 Components of leaf growth

Total shoot leaf area expansion can be dissected into different growth components (Bultynck et al., 2004). The relative growth rate of the whole shoot leaf area is a function of the leaf area expansion rate and the rate at which new leaves and tillers emerge. The leaf area expansion itself depends on the LER, the leaf width and the leaf elongation duration (LED). Bultynck et al. (2004) showed that mainly LER and leaf width is responsible for differences in leaf area and LED to a much lesser extent. LER, leaf width and LED together with leaf area increases with leaf rank on a tiller (Bos and Neuteboom, 1998; Bultynck et al., 2004).

The different components of leaf growth are not completely independent of each other. Studies on a mechanistic basis found independent control of LER and leaf width (Baute et al., 2016; Beemster and Masle, 1996; Beemster et al., 1996; Bultynck et al., 2004). In Maize, LER was indeed found to be largely independent from leaf width (Baute et al., 2016; Lacube et al., 2017). Bultynck Bultynck et al. (2004) found a strong positive correlation ($P < 0.001$, $R^2 = 0.628$) between the two growth components in wheat and wheat related species but the study seems to compare different leaf ranks with each other. All the aforementioned studies state there is no correlation between LER and LED.

It is important to mention that LER and LED are uncorrelated only by comparing genotypes in the same environmental conditions. Many processes appear to be trades-offs between rates and duration especially if not corrected for the influence of temperature (Lacube et al., 2017). High temperatures increase the LER and shorten the LED (see section: 2.1.5 growth control). Comparing plants at different temperature would lead to a negative correlation between LER and LED. However, by correcting for the effect of temperature, LER can be expected to be largely independent of LED. Temperature corrected LER was found to be correlated with final leaf length, leaf area, leaf weight and shoot weight (Baute et al., 2015; Baute et al., 2016). This opens the way for breeding on high LER which ultimately leads to a larger leaf area.

Zhang et al. (2015) found a positive correlation between leaf length and leaf width with biomass. The variance of the two components decides which has the larger influence on leaf area. Several studies in Maize identified leaf width to be the crucial factor, explain a larger part of variance of the leaf area (Lacube et al., 2017; Wei et al., 2016; Yang et al., 2016a). This is contrary to a study in wheat where flag leaf area was mainly influenced by the leaf length indicating differences between the two species (Yang et al., 2016b). Zhang et al. (2015) showed for both, leaf width and leaf length a negative correlation between the number of leaves and tillers. However, the recurrent selection for wider seedling leaves in the same study still resulted in wheat lines producing more than double the seedling leaf area compared to the most vigorous commercial wheat varieties.

2.1.5 Growth control

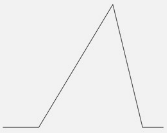
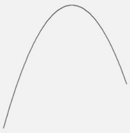
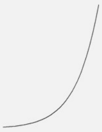
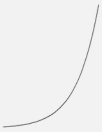
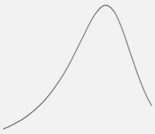
Plant growth can be dissected into endogenous growth patterns and genotype specific responses to environmental conditions (Poiré et al., 2010). Both, the internal growth pattern as well as the response to environmental conditions are genetically determined. Growth control is different between dicot and monocot species. The diurnal growth cycle of dicot species is largely determined by the endogenous rhythm with minor influence of the environmental condition whereas in monocots circadian-clock-controlled processes are largely negligible (Walter et al., 2009).

Environmental factors influence leaf growth by affecting rates of cell division in the meristematic growth zone and affecting cell expansion for example by changing the cell-wall mechanical properties (Ben-Haj-Salah and Tardieu, 1995). The conditions at the meristematic growth zones can largely deviate from the ambient conditions. Especially in monocots, the meristems are located in proximity to the ground and are partly shielded by the leaf sheath. Both factors buffer the meristem temperature which ultimately affects the growth rate (Walter et al., 2009).

The recent advances in ecophysiological modelling of leaf growth in monocots can be summarized by three main findings: (i) LER is mainly controlled by four environmental variables, namely temperature, soil water deficit, evaporative demand and light (Ben (Ben-Haj-Salah and Tardieu, 1997; Bos et al., 2000; Mahdid et al., 2011; Parent et al., 2010b; Salah and Tardieu, 1996). (ii) No appreciable time lag in response of LER to environmental conditions was observed. LER respond to environmental conditions within few minutes (Lacube et al., 2017). (iii) Leaf widening and leaf elongation are distinctly controlled by different environmental variables (Lacube et al., 2017). In the following we go through the major environmental factors.

Temperature has a major influence on growth and largely explains diurnal and long-term changes in LER (Ben-Haj-Salah and Tardieu, 1995; Slafer and Rawson, 1994). The response can be triggered by signalling pathways (Franklin, 2009; Penfield, 2008) or simply be result of the temperature dependant kinetics of biochemical reactions (Parent et al., 2010a). The effect of temperature on LER was found to be

Table 1: Overview of the major equations used to describe leaf elongation rate (LER) as a function of temperature.

Name	Equation		Application	Plot	Example paper
Linear equation	$LER = a(T - T_0)$	(Eqn. 1)	Traditional version of Thermal time		Sadok et al. (2007), Reymond et al. (2003)
Bilinear equation	$for\ T_b < T < T_{opt}$ $LER = a(T - T_0)$ $for\ T_{opt} < T < T_m$ $LER = (T_m - T)\frac{a(T_{opt}-T_b)}{T_m-T_{opt}}$	(Eqn. 2)	Crop models		Hammer et al. (1994), Hammer et al. (2010)
Quadratic equation	$LER = a_0 + a_1T + a_2T^2$	(Eqn. 3)	-		Sadok et al. (2007)
Exponential equation	$LER = a + be^{kT}$	(Eqn. 4)	-		Peacock, (1975)
Arrhenius equation	$LER = Ae^{-E_i/(RT_{^\circ K})}$	(Eqn. 5)	Chemical reactions		Sadok et al. (2007)
Eyring equation	$LER = ATe^{\left(\frac{\Delta H_A^{\ddagger}}{RT}\right)}$	(Eqn. 6)	Biochemical reactions in lower temperature range		Eyring (1935), Parent et al. (2010)
Johnson equation	$LER = \frac{aTe^{\left(\frac{\Delta H_A^{\ddagger}}{RT}\right)}}{1+e^{\left[\frac{\Delta S_D}{R}\left(1-\frac{\Delta H_D}{\Delta S_D T}\right)\right]}}$	(Eqn. 7)	Biochemical reactions in entire temperature range		Johnson et al. (1942), Parent et al. (2010)

reversible, meaning that LER at a given temperature is independent from previous temperatures (Parent and Tardieu, 2012). Temperature affects both leaf elongation and leaf widening by changing the rate but without appreciable influence on final dimension of leaf area (Parent and Tardieu, 2012, Lacube et al., 2017). There are several equations used in literature to describe the influence of temperature on LER (Table 1).

Parameter values for some of the equations in Table 1 can be found in literature. Porter and Gawith (1999) reviewed 65 papers and reported base, optimum and maximum temperatures for different growth and elongation processes. Leaf initiation and shoot growth were maximal at 22.0°C and 20.3°C, respectively. Nagai and Makino (2009) found the highest relative growth rates (RGR) in the temperature range of 19 to 25°C. Linear growth response to

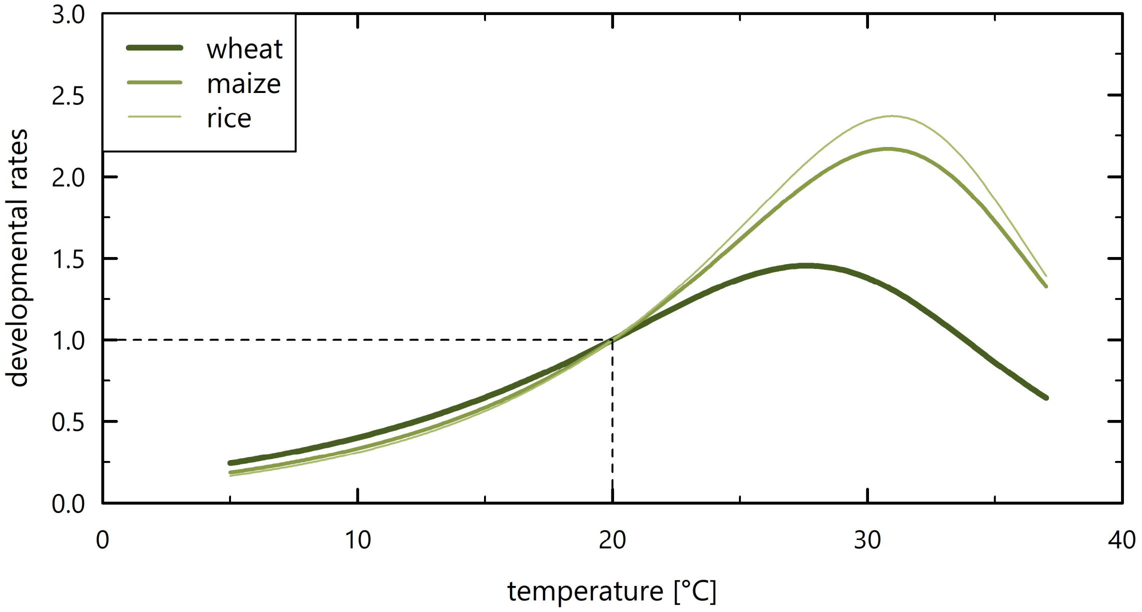


Fig. 4: Normalized temperature response (divided by rate at 20°C) is largely congruent across of a large range of developmental processes and across contrasting genotypes within one species. The Figure presents the normalized response curve of wheat, maize and rice fitted by the Johnson equation (Johnson et al. 1942). Parameter values were taken from Parent et al. (2012).

temperature (Eqn. 1) in maize was analysed in Reymond et al. (2003) and in Sadok et al. (2007b). In Reymond et al. (2003) the slope of the linear response ranged between 3.3 to 5.7 mm°C^{-1}d^{-1}. Both studies found the slope to be a stable characteristic of a certain genotype. This is also supported by Grieder et al. (2015) which found stable genotype-specific linear responses of temperature on RGR in wheat.

The appropriateness of the individual equations depends on the plant species and the temperature range in which the plants are grown. Parent et al. (2010a) for example, found the linear equation to be appropriate within a relatively wide temperature range for Maize and Arabidopsis, but not appropriate for rice. Therefore, the study recommends using the generally applicable equation of Johnson et al. (1942) to estimate the thermal effect on LER. The equation successfully describes the temperature response of various developmental processes like germination rate, cell division rate, leaf initiation rate, LER, and the reciprocal of the duration of phenological phases and was tested successfully in 17 crop species (Parent et al. 2010a, Parent and Tardieu, 2012). The normalized equation (divide by rate at 20°C) was similar for all developmental processes and almost indistinguishable between varieties with diverse breeding histories and contrasting target environments. The results imply a common normalized temperature response curve for all wheat varieties with the same optimum temperature (Fig. 4). The only difference between varieties would be the absolute values which can be obtained by scaling the normalized values with the genotype specific scaling parameter *a* (Table 1)

Evaporative demand and **soil water deficit** are both water-related environmental variables with a large effect on LER. Cell and tissue expansion immediately react on dehydration and are the first processes affected by water shortage (Muller et al., 2011; Tardieu, 2013). The reason for that might be the central role of water in growth processes. Water deficit leads to a reduction in turgor and has negative effect on cell division rate and the extensibility of cell walls (Cosgrove, 2005; Tardieu, 2013). Soil water deficit and evaporative demand which both negatively affect leaf water potential have therefore a negative effect on LER. Water availability is also affected indirectly via root temperature. The hydraulic conductivity of roots decreases with decreasing temperature. Root cooling has an immediate effect on LER as shown by Malone (1993).

The sensitivity of LER to water deficit is controlled mainly by hydraulic mechanisms (by decreasing leaf water potential in the leaf growing zone) and not so much by chemical signals (Tardieu et al., 2014). This leads to a relatively short reaction time of less than 30 min (Caldeira et al., 2014). The sensitivities of LER to soil water deficit and to evaporative demand is species and genotype specific (Reymond et al., 2003). Response of LER was found to be linear in a large range for VPD (0-6 kPa)(Ben-Haj-Salah and Tardieu, 1997; Reymond et al., 2003) and soil water potential (0-1 MPa), (Reymond et al., 2003; Welcker et al., 2011). In addition to the influence on LER, evaporative demand was shown to have a negative effect on final leaf length in Maize (Lacube et al., 2017).

Light is expected to have a positive effect on leaf width but a negative effect on final leaf length and LER. Lacube et al. (2017) for example found a close relationship between final leaf width with the cumulative intercepted light per plant in the period with maximum widening rate. These results are in contrast to Bos and Neuteboom (1998) which found no influence of light on LER and on leaf width in spring wheat. The mechanisms behind the possible effect on leaf width are rather unclear. The possible effect on LER might be linked to hydraulic influence as light has a negative effect on leaf water potential (Ben-Haj-Salah and Tardieu, 1996). The coordinated response of several growth-related physiological processes to UV indicate a controlled adaption rather than direct photochemical damage (Wargent et al., 2009).

Temperature, evaporative demand, soil water deficit and light are key factors in explaining the short-term variation of LER. They are also very important in explaining long term differences between experiments, however, several other factors might also have an influence on LER in the long term. Soil resistance to root penetration is closely connected to soil hydraulic properties and was found to have a large effect (-50%) on LER (Beemster et al., 1996). Plant nutrition has a large effect on growth therefore it might not be surprising that phosphorus deficit lead to a decrease in leaf elongation rate by affecting the rate of cell division and cell elongation simultaneously (Kavanová et al., 2006). A study in Maize found a positive influence of high plant density on LED in lower leaf ranks. LER was almost unaffected during the phase of linear extension rate, with only small effect on leaf 11 (Andrieu et al., 2006).

It is not hard to imagine that LER will respond to all kind of biotic and abiotic stressors which somehow influence carbon assimilation and partitioning as well as the hydraulic status of the plant. Therefore, absolute LER values might not be reproducible across studies in different environments, whereas differences in LER between varieties might be.

2.1.6 Genetic control

Leaf growth at the cellular level is well described but the molecular mechanisms are still insufficiently explained. A simple molecular description of leaf growth is impossible due to (i) the large number of processes involved in leaf growth (Nelissen et al., 2016), (ii) the large influence of environmental factors on leaf growth (Walter et al., 2009), (iii) the complex polygenetic control of leaf growth with alleles distributed throughout the genome (El-Lithy et al., 2004), (iv) the large G×E interaction of expansive growth (Sadok et al., 2007a).

To disentangle the overriding effect of environment and G×E interaction on intrinsic elongation rate under favourable conditions, (Sadok et al., 2007a) proposed a combined approach of environmental modelling followed by the genetic dissection of the model parameters. The concept was successfully applied by Sadok et al. (2007b) and Reymond et al. (2003). The effect of QTLs found by Reymond et al. (2003) was confirmed by Sadok et al. (2007b). However, QTLs were not stable in different mapping populations. Reducing the number of QTLs to a smaller number of meta-QTLs might be one way to improve the detection of stable QTLs, like it was shown for the effect of VPD and Soil moisture deficit on LER (Welcker et al., 2011). The independent control of leaf width and leaf length was also confirmed on molecular level, with separate and independent QTLs for the two traits (Lacube et al., 2017).

Another approach to detect genes associated with LER are transcriptome profiling studies. The expression level of 226 genes were correlating with at least one growth related trait in in two populations of Maize (Baute et al., 2016). However, also here a very small percentage of those genes were found to correlate with LER in both populations. Most growth-related genes were associated with the functional categories regulation of transcription, protein synthesis and cell wall synthesis and degradation and influence growth as part of a holistic growth regulatory network.

2.1.7 Modelling LER

A widely used equation (Reymond et al., 2003; Sadok et al., 2007b) estimates LER based on the linear effect of temperature, VPD and soil water deficit

$$LER = (T - T_0)(a + b\,VPD + c\,\psi) \quad \text{(Eqn. 8)}$$

where T is the meristem temperature, T_0 is the x axis intercept of the temperature response and a, b and c are the slope of the LER response to temperature (T), VPD and soil water potential (ψ) respectively. The effect of light was already included by adjusting the value of VPD accordingly. This equation is expected to hold true only in a limited range of environmental conditions. For an implementation in crop models a larger range would be preferable. In an extended range, growth reaction to environmental conditions are often highly nonlinear (Walter et al., 2009). Fitting robust nonlinear models for plant growth remains challenging (Paine et al., 2012). A stepwise modelling might help to disentangle the effect of the different environmental parameters. Especially the dominating effect of temperature can obscure developmental trends or the effects related to hydraulic conditions (Parent et al., 2010a).

Thermal time has been used in crop modelling for more than 30 years to express durations of phenological stages temperature independently. Also, it has recently been used to express physiological rates in a temperature independent manner (Sadok et al., 2007b). In the traditional version, thermal time is calculated on a daily basis as the product of time and temperature exceeding the minimum temperature limit of growth (T_0). Sadok et al. (2007b) were the first to successfully use thermal time on a time scale of 15 minutes to express LER temperature independent. The temperature range for valid application of thermal time can be expanded by accounting for the non-linear effect of temperature with the Johnson equation (Parent et al., 2010a). The temperature independent expression of LER opens the way for analysing the effect of phenological stages and additional environmental factors, namely water related factors, on LER.

The physiological model to predict LER can be extended by a genetic model to predict environmental response curves based on QTL effects. It allows a prediction of LER in genotypes with undetermined environmental response curves. Reymond et al. (2003) predicted 74% of the LER variability in Maize RILs based on QTLs influencing the slope of the response curve on temperature, VPD and soil water deficit. Predictions like those could be used directly for selection in plant breeding or for yield predictions in crop models. LER models are a key component of crop models. Improvements in predicting leaf area could significantly improve accuracy of crop models (Martre et al., 2015).

2.1.8 Leaf growth and yield

Growth is a key process in yield formation. Growth rate together with growth duration determines the final biomass of the plant. Biomass is directly transformed into yield, whereby the harvest index determines the efficiency of this process (Diepenbrock et al., 2009). Leaf growth is of special importance as it increases the amount of intercepted light which ultimately effects carbon assimilation (Tardieu, 2013). Especially flag leaf area has been shown to be strongly linked with grain yield (Yue et al., 2006).

High growth rates might lead to a rapid early leaf area development which is positively correlated with above-ground biomass and grain yield in wheat (van den Boogaard et al., 1996). Mechanisms involve reduced soil evaporation due to soil coverage, increased uptake of soil nutrient, higher competitiveness against weeds and a higher light interception (Bultynck et al., 2004). The potential water savings are especially valuable in dry Mediterranean climate, where breeding on early leaf area development and vigour is well established (Zhang et al., 2015).

2.2 Water Stress

2.2.1 Importance of Water

Water is of crucial importance for plants (Jackson et al., 2000). Biochemical reactions take place in aqueous solution where water serves as a carrier medium and reactant simultaneously. Osmotic water movement leads to turgor pressure providing stability and enabling cells to expand. Transport of nutrients and assimilates in the vascular tissue is driven by water flow and powered by the transpiration of water through the stomata. The cooling effect of transpiration prevent an overheating of plant tissue. Lack of water threatens these vital functions of a plant, which is why plants have several mechanisms to prevent dehydration.

2.2.2 Interplay of supply and demand.

Plant water content is influenced by water supply from the soil and water demand caused by transpiration (Fig. 5) (Aroca et al., 2012). The supply depends on root traits and soil characteristics give a certain amount and distribution of precipitation over the year. Together they determine the maximum amount of water which could be taken up by plants. On the demand side, transpiration depends on leaf traits and atmospheric conditions. A long-term imbalance between water appropriation and water loss leads to water stress and dehydration. Therefore, it is important for the plant to regulate water uptake and transpiration.

2.2.3 Plant responds

To a limited extent water uptake can be regulated by regulating the root hydraulic conductivity (Vitali et al., 2015). Much more important is the regulation of loss processes. Leaf area, stomatal density and stomatal conductance are all subject to dynamic response on environmental conditions mainly triggered by hydraulic and chemical messages (Buckley and Mott, 2013; Hill et al.,

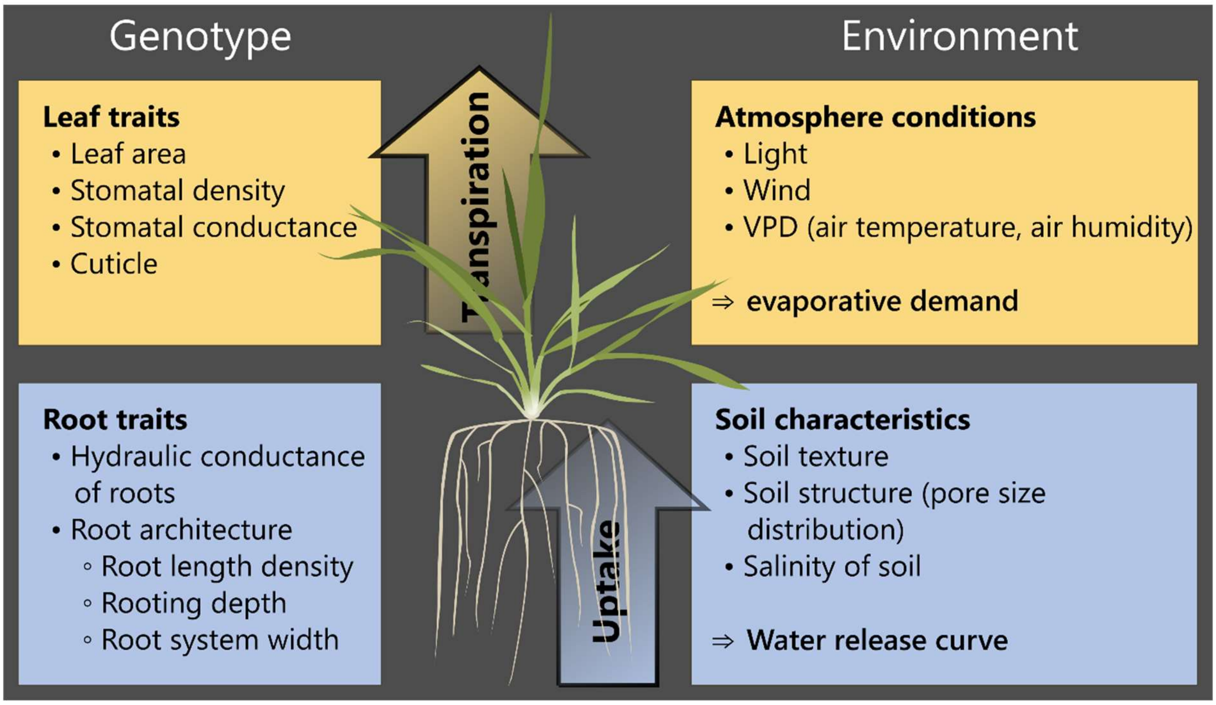


Fig. 5: Factors influencing plant water status given the amount and distribution of precipitation over the year. Separated in genotypic factors (left) and environmental factors (right) as well as in factors reducing transpiration (yellow) and factors increasing water uptake (blue). According to Miglietta et al 2011, Tardieu 2013, Jackson et al. 2000, de Jong van Lier 2014, Aroca et al 2012 and Steudle 2000.

2015; Lacube et al., 2017). However, downregulating transpiration processes comes at a cost. All processes are expected to have a negative influence on photosynthesis and might therefore be negatively associated with yield (for leaf area see chapter 2.1.8, for stomatal density see Tanaka et al. (2013), for stomatal conductance see Roche (2015)). In addition to the water-saving measures, plants react on water stress with a variety of molecular mechanisms to increase stress tolerance and prevent excess light (well described in Osakabe et al. (2014)).

2.2.4 Plant adaptation

The short-term adaptation (response) to water stress are complemented by long term evolutionary adaptation (morphological and physiological) of plants to survive in drought prone environments. The adaptations are often classified into four strategies (Fig. 6) (Izanloo et al., 2008; Tardieu, 2005): (i) drought escape where plants finish their live cycle before severe water stress damages plant functioning, (ii) drought avoidance which include all measures to increase water uptake and decrease transpiration which also include short-term adaptation described above, (iii) drought tolerance where plant physiology is adapted to allow growth under

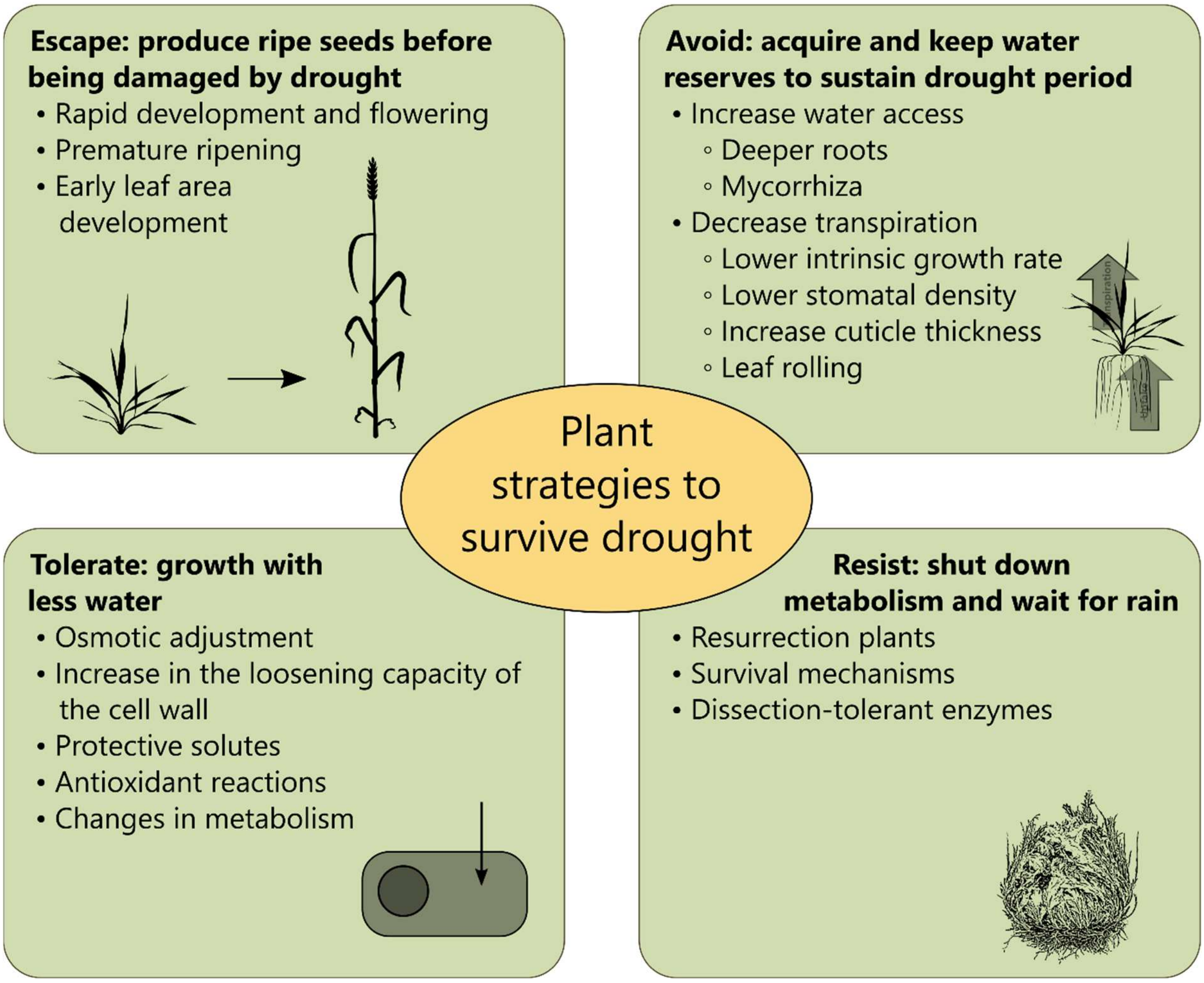


Fig. 6: Four plant strategies to survive drought stress. For each strategy some examples are given. According to Tardieu 2013, Izanloo et al. 2008, Bodner et al. 2015, Yousfi et al. 2015, Stagnari et al. 2014, Saab 1992, Hsiao and Xu 2000

water stress and (iv) drought resistance which describes mechanisms to survive almost complete dehydration.

2.2.5 Yield maintenance

Of several yield limiting factors in wheat, water stress is the most important one (Tester and Langridge, 2010). The effect can be quantified with the yield response factor (K_y) introduced by the FAO (Doorenbos and Kassam, 1979):

$$1-\frac{Y_a}{Y_x}= K_y\left(1-\frac{ET_a}{ET_x}\right) \qquad \text{(Eqn. 9)}$$

where Y_a and Y_x are the actual and maximum yields and ET_a and ET_x are the actual and maximum evapotranspiration. K_y quantifies the effect of reduced evaporation due to water stress on yield. It is directly linked to WUE which defines the increase in biomass per transpiration in one day (Tardieu, 2013). The effect is not constant over crop cycle and depends on the phenological stage. Stages like flowering and yield formation have been found to be highly susceptible to water deficit whereas water stress during ripening and vegetative phase have less impact on wheat yield (Zhang and Oweis, 1999). Deficit irrigation make use of this knowledge by saving irrigation water in uncritical stages (Geerts and Raes, 2009). In addition to technological measures, breeding represents a suitable method to decrease yield penalties under water stress (Tester and Langridge, 2010). The breeding efforts can be categorized into the four strategies presented in Fig. 6.

The escape strategy is well adopted in many wheat breeding programs. Several studies show a 10-13 days shift towards early flowering within one decade of wheat, bread for environments with frequent terminal drought (Shavrukov et al., 2017). The strategy adapts the crop cycle to be better synchronized with high water availability and low evaporative demand. However, breeding on drought escape often reduces the duration of the cycle which might include a reduction in yield potential (Tardieu, 2013). Therefore, breeders face a trade-off between lower risk of terminal stress against reduced yield potential.

The avoidance strategy includes all breeding effort to increase water uptake and reduce transpiration. Adaptations in root architecture which lead to an expansion of the root area exploit previously unused water resources in deep soils. However, in soils with root barriers (physical or chemical) which limit soil volume, breeding on improved yield under water deficit resulted in a reduction of root biomass (Bolaños et al., 1993; Bruce et al., 2002). A large rooting system would deplete limited soil water reserves more rapidly and be disadvantageous. The reduction in transpiration is again a trade-off between photosynthesis and lower risk of terminal stress (Tardieu, 2013). Reducing the nighttime transpiration is highly promising, as photosynthesis is unlikely to be affected (Coupel-Ledru et al., 2016; Schoppach et al., 2016).

Drought resistance has limited potential for agriculture as it addresses the problem of survival rather than the problem of production (Tardieu, 2005). The ability to stop metabolism almost completely for months is only agronomically interesting in very extreme environmental conditions. Nevertheless, there is research going on trying to upregulate genes in maize which have been found to be involved in drought resistance of resurrection plants (Farrant et al., 2015).

Finally, drought tolerance represents a one of the most promising option to deal with water stress. According to Reymond et al. (2003) a plants drought tolerance can be estimated by measuring its growth response to water deficit (soil water deficit or VPD). Plants with steepest response immediately save soil water and maintaining leaf water potential by stopping growth. Drought tolerant plants with low response keep growing and adapt their physiology to maintain photosynthesis. Active photosynthesis requires open stomata and expansive growth increases the leaf surface. Both together lead to increased water consumption compared to drought sensitive varieties and increase the risk for terminal stress. Which strategy is now more efficient? There is no single answer to this question because it depends on the drought scenario (Tardieu, 2013). A severe drought which greatly threatens the survival of a plant favours drought sensitive species as they are more likely to survive the long period. Drought tolerant plants perform better in a mild drought scenario as they keep growing, accumulate more biomass and finally have higher yields. This hypothesis based on theoretical considerations was confirmed by field trials and crop modelling (Chenu et al., 2008; Chenu et al., 2009). (Tardieu and Tuberosa, 2010) therefore propose a twostep approach in breeding. First drought tolerance is measured for candidate varieties based on growth response to water deficit of predicted based on major effect QTLs or genomic prediction. In the second step, crop models are used to assess the yield performance of a candidate variety in a certain drought scenario. This allows selecting for varieties with high performance in specific current or future drought scenarios.

3. Materials and Methods

3.1 Genetic material

Genetic material consisted of the GABI-wheat panel (Kollers et al., 2013), a diverse panel representing the last 50 years of wheat breeding in Europe. The panel used here includes 320 winter wheat (WW) and 12 spring wheat (SW) varieties. The large majority are varieties from German and France breeders but also Swiss varieties are well represented (Table 2). All varieties were registered between 1948 to 2014 on national variety lists. The diversity of the mapping population could help to find QTLs which are more robustly associated with a trait and reproducible in most wheat varieties (Baute et al., 2016).

All varieties were genotyped with a 90k Infinium chip (iSELECT, San Diego, USA) prior to this study. The entire genotyping and association analysis part is described in (Yates et al., 2018).

Table 2: The composition of the GABI wheat panel from varieties of different European countries.

Country	Number of varieties
DE	103
FR	89
UK	30
PL	28
CH	24
DK	14
AT	13
SE	9
CZ	5
–[a]	27

[a] Origin is unclear

3.2 Experimental set-up

Wheat (*Triticum aestivum* L.) plants were grown in a greenhouse at Zurich, Switzerland (47°25'41"N 8°31'00"E) between April to September 2018. Plants were sown in pots (75 × 75 mm area and 85 mm height) containing roughly 85 g dry weight substrate. The substrate ("Containererde für Stauden und Kübelpflanzen", Ökohum gmbh, Herrenhof, Switzerland) was an 84:16 (v/v) mixture of organic components (compost, peat, wood fibres) and mineral components (clay, sand). The clay had an aggregate diameter between 2 to 10 mm and the sand particle size were ranging from <0.1 to 4 mm. Ten seeds were sown per pot (corresponding to a seed density of 1780 seeds m^{-2}) at 10 mm depth. The high seed density should lead to a high transpiration and thus a rapid dehydration of the pot.

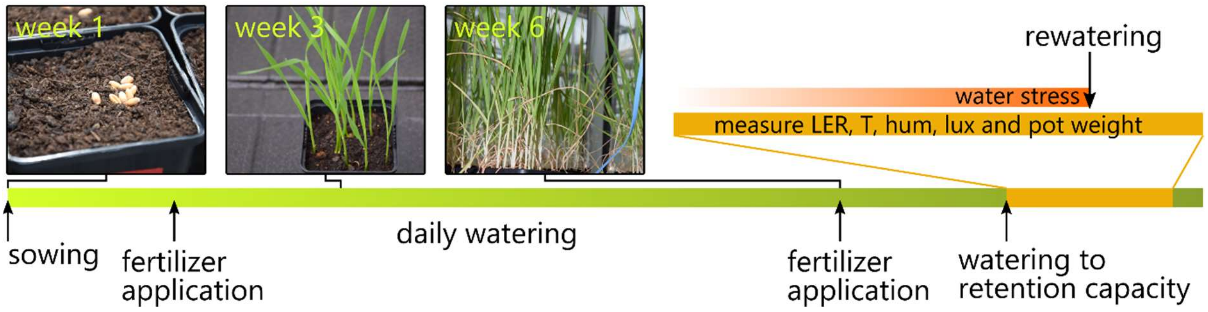


Fig. 7: Timeline of the experiment. Plants were daily watered during the first 6 weeks in the nursery. After week 6, plants were transferred to phenotyping platform and kept without watering for 4 to 6 days.

Fig. 8: Soil water deficit and leaf elongation rate tracking on phenotyping platform Monocot Envirotyping Unit. (a) 10 Wheat plants at leaf stage 6 ready to be measured on the phenotyping platform. (b) Pots are placed on a scale for a continuous measurement of soil water deficit. (c) Youngest leaf of one plant per pot is connected via a hair clip and a thread with (d) a rotating wheel and a counterweight. (e) Wheel rotates along with a magnet. The orientation of the magnetic field is measured with a magnetic field sensor. Each change in angle is associated with change in leaf length.

Plants were grown six weeks in a nursery and then transferred to the phenotyping platform where leaf growth was measured for one week (Fig. 7). At the end of the week, final leaf length was measured from leaf tip to the ground. In the nursery, plants were daily watered by flooding the table for 30 min. Temperature was influenced by the outside temperature and ranged between 8 to 34°C. High-pressure sodium lamps were used to extend the day length from 6:00 to 22:00. The lamps were automatically turned off at a certain sunlight intensity (>500 Wm^{-2}) and air temperature (>23°C). Liquide fertilizer was applied at week 1 and week 5 containing all important plant nutrients (Wuxal Universaldünger, Maag/Syngenta, Dielsdorf, Switzerland). Macronutrient content of one fertilizer application was equivalent to 1’780 kg N ha^{-1}, 1’780 kg P_2O_5 ha^{-1} and 1’330 kg K_2O ha^{-1} which should prevent a nutrient deficiency in any case. Just before transfer to the phenotyping platform, soil was watered to retention capacity.

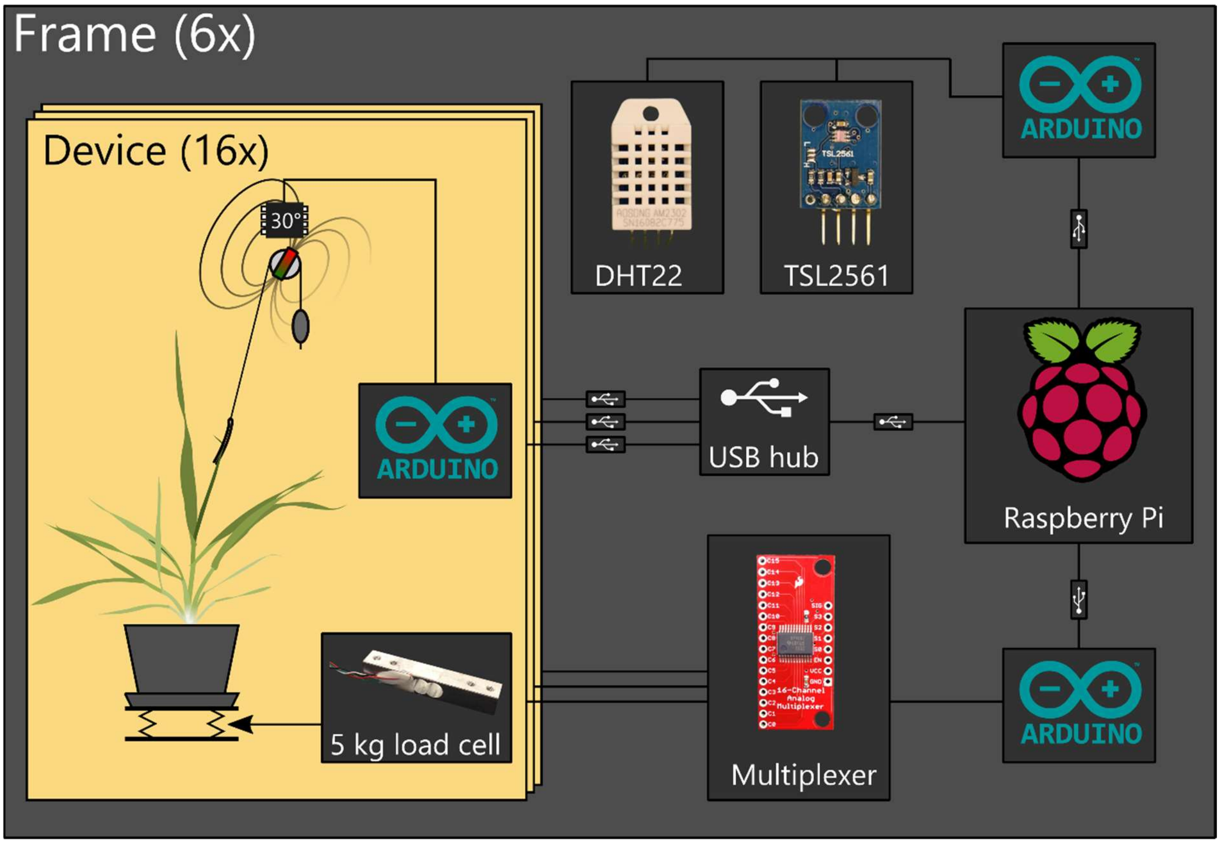


Fig. 9: Phenotyping platform Monocot Envirotyping Unit. One frame consists of 16 devices each with a leaf tracker and a 5 kg load cell scale. The leaf trackers are connected via USB hub with a Raspberry Pi. The signal of the scales is merged in one cable by a multiplexer and connected via an Arduino to the Raspberry Pi. The DHT22 measures temperature and humidity and the TSL2561 measures illuminance both for the entire frame. The sensors are controlled by an Arduino. All Arduinos are finally connected to the Raspberry Pi which acts as data logger. The phenotyping platform consist in total of six frames as illustrated here.

3.3 Phenotyping platform

The monocot envirotyping unit (MEU) is a phenotyping platform designed to measure the effect of environmental factors on LER (Fig. 8). 96 pots each containing ten plants of one genotype were measured at the same time, continuously over one week. The pots were evenly distributed between three chambers, each having two frames with 16 measuring devices (Fig. 9).

Each pot was placed on a scale (5 kg load cell with analog-to-digital converter HX711, Avia Semiconductor Co. Ltd., Xiamen, China) to measure changes in soil water status. Scales were highly sensitive to changes in temperature and humidity. The following multiple linear regression model was used to estimate a linear response curve during calibration process (Fig. 10):

$$m = i + s\,SV + a\,T + b\,\varphi \qquad \text{(Eqn. 10)}$$

Where m is the mass, i is the intercept, SV is the digital scale value, T the temperature, φ the relative air humidity and s, a and b the slope of the response to these factors. After correcting weight for temperature and humidity, scales had an average standard deviation of 3.07 g. The signal of 16 scales was gathered by a multiplexer (CD74HC4067, Texas instruments, Dallas, USA) and processed by a microcontroller (Arduino nano, Arduino, Turin, Italy).

In order to measure LER, the youngest leaf (normally leaf 6, sometimes leaf 5 or 7) of one plant per pot was connected to a pulley via a hair clip and a thread. A counterweight

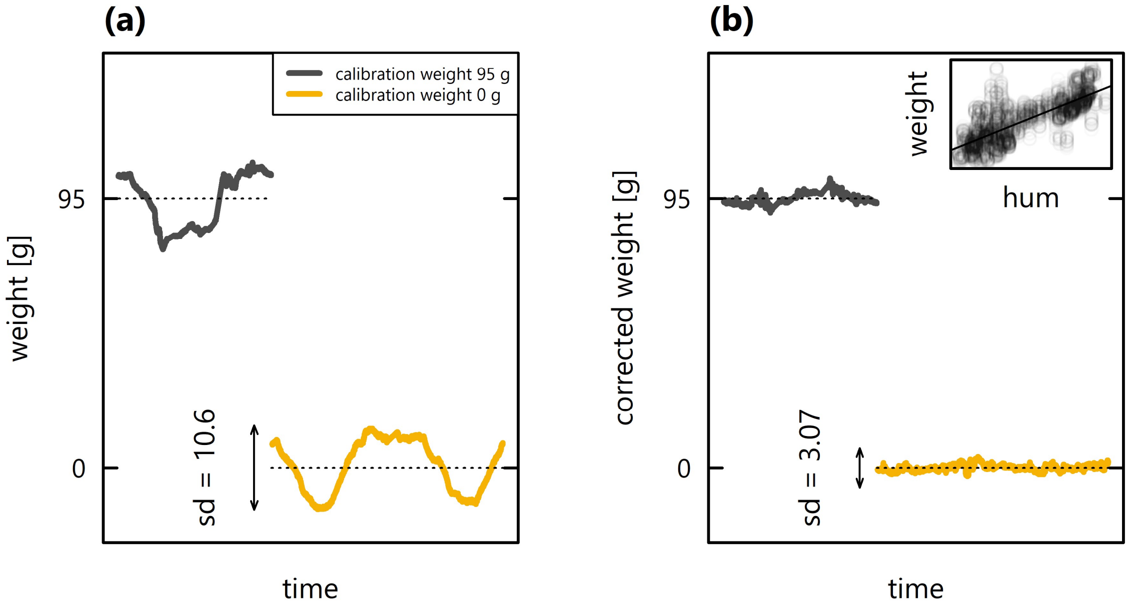


Fig. 10: Calibration of scales. (a) Calibration based on calibration weights without considering effect of temperature and humidity. Scale weights are distributed around desired value and follow the diurnal cycle of temperature and humidity. Standard deviation (sd) of measured minus actual weight is high. (b) Calibration with considering a linear effect of temperature and air humidity on scale value. Sd can be reduced considerably. (b) inset, linear relationship between humidity and scale weight.

(20 g) attached to the thread on the other side of the pulley ensured constant tension of the thread. Changes in the leaf length led to a rotational movement of the pulley. The pulley had a magnet inserted through the shaft. The orientation of the magnetic field was measured by a magnetic rotary position sensor (AS5600, AMS, Premstätten, Austria) attached to a microcontroller (Arduino micro). For each measurement, the magnetic rotary position sensor measured 100 times and the average was reported. The constant pulling force of the 20 g counterweight has neglectable or no influence on LER some hours after leaf emergence as shown in several independent experiments (Ben-Haj-Salah and Tardieu, 1995; Gallagher et al., 1976; Parent et al., 2009; Sadok et al., 2007b; Walter et al., 2002). Effect of temperature and humidity variation on thread length were tested separately. The effect of humidity was significant ($p < 0.001$) with an estimated effect of 0.0022 mm $\%^{-1}$ humidity. Humidity variation between 0 and 100% would lead to a maximum thread length difference of 0.22 mm which is neglectable.

Environmental conditions were measured for each frame separately. Temperature and humidity were measured (DHT22, Adafruit Industries, New York, USA) as well as illuminance (TSL2561, AMS). The sensors were connected to an Arduino nano microcontroller and placed at the same height as plant meristems within the plant canopy.

All the Arduino microcontroller on one frame were connected to a single-board computer (Raspberry Pi 3 Model B, RS Components Ltd., Northants, UK) via three USB hubs. Measurement data were saved on an SD card every 42 s, 2 min and 17 min for scale data, environmental data and LER data, respectively. Regular disconnection of USB devices most probably due to power drain or bandwidth exceedance caused large data

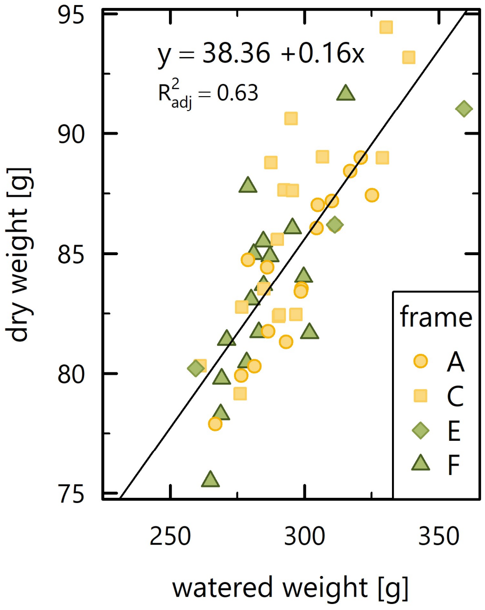


Fig. 11: Linear Regression between soil weight watered to retention capacity and soil dry weight. The regression line allows a prediction of the soil dry weight based on pot weight at start of experiment.

gaps. To avoid very long data gaps, the computer automatically soft rebooted every hour and was disconnected from power every six hours. The latter always led to a successful reconnection with the microcontrollers.

3.4 Estimating plant water status

Substrate of 51 pots was oven-dried after the experiment. The dry weight was correlated with water saturated pot weight at start of experiment:

$$DW = i + m\,SSW \qquad \text{(Eqn. 11)}$$

Where DW is the dry weight, i is the intercept, m is the slope and SSW is the saturated soil weight at start of experiment. The resulting regression line (Fig. 11) was used to estimate dry weight of all pots in the experiment. Gravimetric water content of each pot during the measuring week was calculated with the following equation:

$$GWC = \frac{SW - D}{DW} \qquad \text{(Eqn. 12)}$$

Where SW is the soil weight and DW the estimated dry weight from Eqn. 11.

Gravimetric water content is not informative of water availability of the plant. Therefore, the water content needed to be transformed into water potential using a substrate specific pF curve. Nine substrate samples were taken from different pots and used to estimate the pF curve of the substrate. The samples were placed on a tension table (pF-Laborstation, ecoTech Umwelt-Meßsysteme GmbH, Bonn, Germany) to measure water content at 100 hPa. Afterwards, the samples were transferred to a pressure plate (5 bar pressure plate extractor, Soilmoisture Equipment Co. Santa Barbara CA, USA) were pressure was increased to 1'000 hPa. Finally, samples were oven-dried and dry mass was measured. In order to estimate the dry end of the pF curve, the same method as described in Tuller and Or (2005) was used. 17 substrate samples were dried out and rewatered to a range of 0 to 50% volumetric water content. Each sample was mixed and stored in plastic cups within Ziploc bags to prevent evaporation and to allow equilibration of the water within the sample. After two days, the water potential of each sample was measured with a dewpoint potentiameter (WP4-T, Decagon, Pullman WA, USA). To determine the precise gravimetric water content, samples were weighted then dried for two days and weighted again. Finally, all data from different measuring devices were combined to estimate the pF curve of the substrate (Fig. 12).

The increase in mean plant biomass over one week was expected to be neglectable compared to total pot weight. Above ground

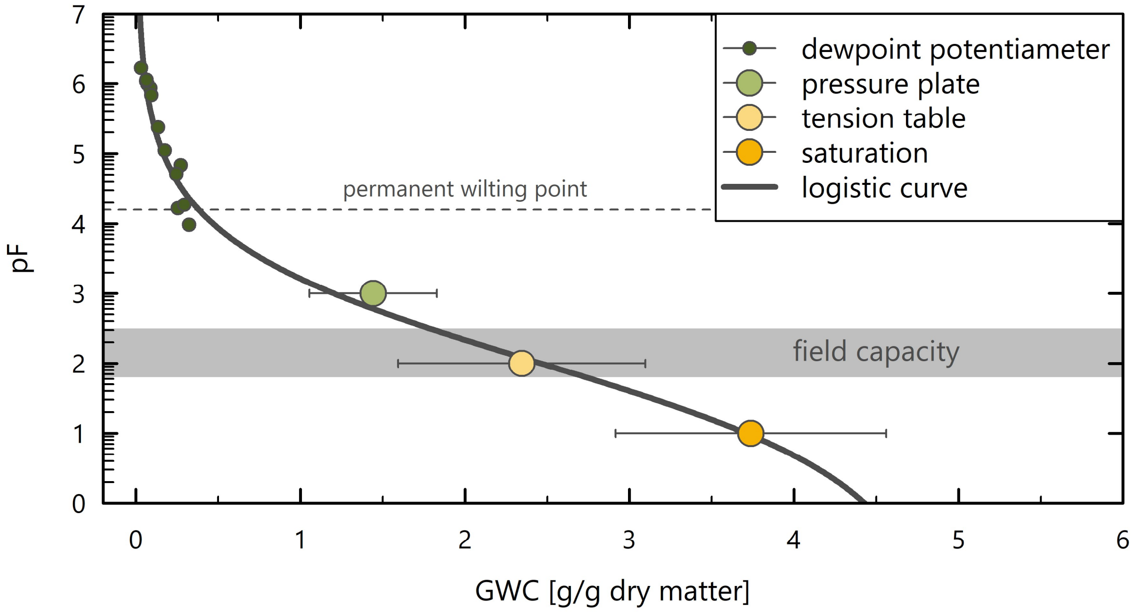


Fig. 12: pF curve. The curve was estimated using different methods for the wet end and the dry end as shown in the legend. Pots at retention capacity at the start of the experiment had GWC values between 2.5 to 3 g/g dry matter which is slightly above the estimated field capacity. Almost the entire soil water is plant available. Only about 10% of the initial soil water at the start of the experiment is kept in the soil at a soil water potential lower than -$10^{4.2}$ hPa (permanent wilting point).

plant fresh weight only accounted for 1.5% of water saturated soil weight.

3.5 Manipulation of environmental conditions

Throughout the experiment we sought to maintain temperature in a range of 5 to 40°C (Fig. 13) with a typical diurnal cycle. The start of increasing daytime temperature was delayed, allowing a better statistical separation between effect of temperature and light. For the same reason, humidity was increased on Saturday and Sunday between 17:00 and 18:30 as well as at an irregular schedule during the week with an air humidifier (Defensor Type 3001, Condair, Freienbach, Switzerland) and by spraying water on the floor. High-pressure sodium lamps were activated between 12:00 to 22:00 in case of low sun light intensity. For the third replicate, artificial light was completely omitted. All these measures were a means to

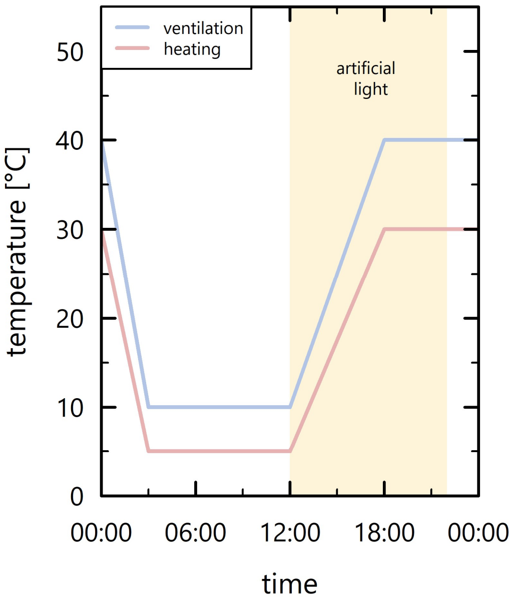


Fig. 13: Temperature regime on the phenotyping platform. Greenhouse was heated if temperature fall below heating temperature and ventilated if temperature exceeded ventilation temperature.

increase variance of temperature, humidity and light and independence between them.

Water was withheld for 4 to 6 days to simulate drought conditions (Fig. 7). Due to the small pots and the high planting density, low soil water potentials were reached within a short time. After simulating drought conditions, plants were rewatered with approximately 200 ml of water.

3.6 Experimental design

Measuring one leaf over a week was considered as one replicate. Each of the 320 genotypes were measured with three replicates making it in total 960 measurements of one week. The phenotyping platform offers 96 measuring devices minus one which was used for the check variety (CH CLARO). Therefore, measuring all genotypes of one replicate took four weeks and the remaining devices in the last week were filled up with genotypes of the next replicate. Genotypes were randomized within one replicate. In the following case the randomization was adjusted by flipping two genotypes: (i) One genotype was randomly distributed to a device which already measured the genotype once. The flipping should prevent a systematic bias of one genotype due to the measuring devices. (ii) Two replicates of the same genotype were randomly assigned to the same week in which the replicates overlap. In such a case, the genotype would face much less variation in environmental conditions which would negatively affect the model predictions.

3.7 Data analysis

The magnetic angle data was converted to leaf length and the differences between abs leaf length was used as LER. The LER values were combined with environmental data (temperature, humidity, illuminance) and scale data by averaging all environmental and scale data between two successive LER measurements and assigning them to the

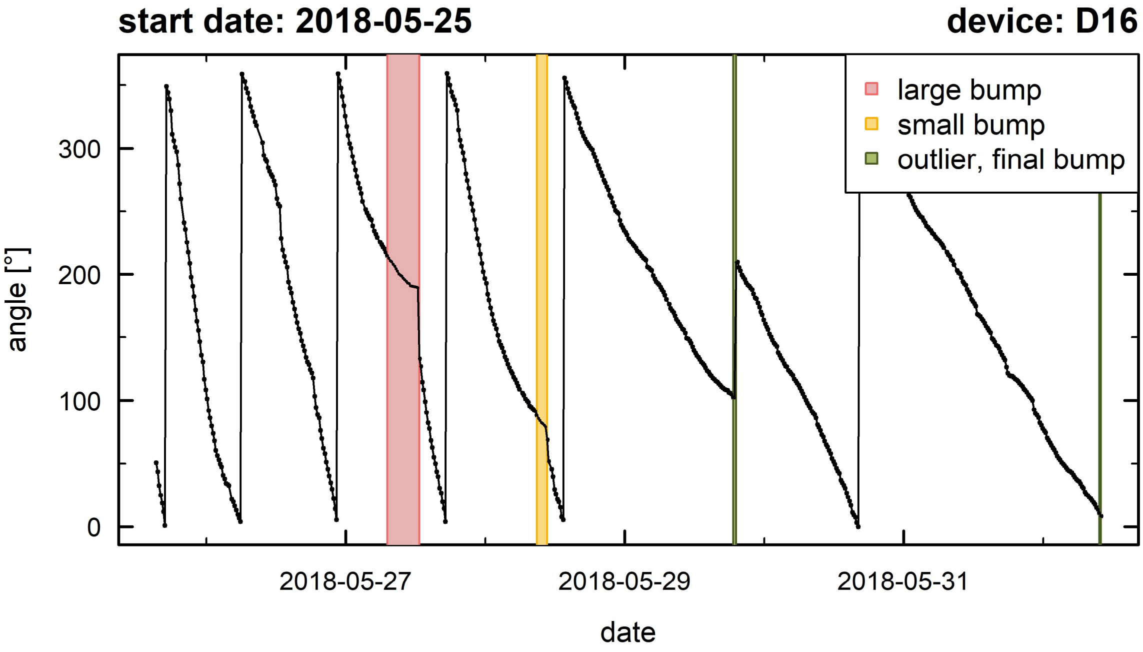


Fig. 14: Time course of magnetic angle data showing unsmooth rolling of the pulley. Changes in angle were slowing down and sometimes stopped completely. These data needed to be detected and removed from analysis.

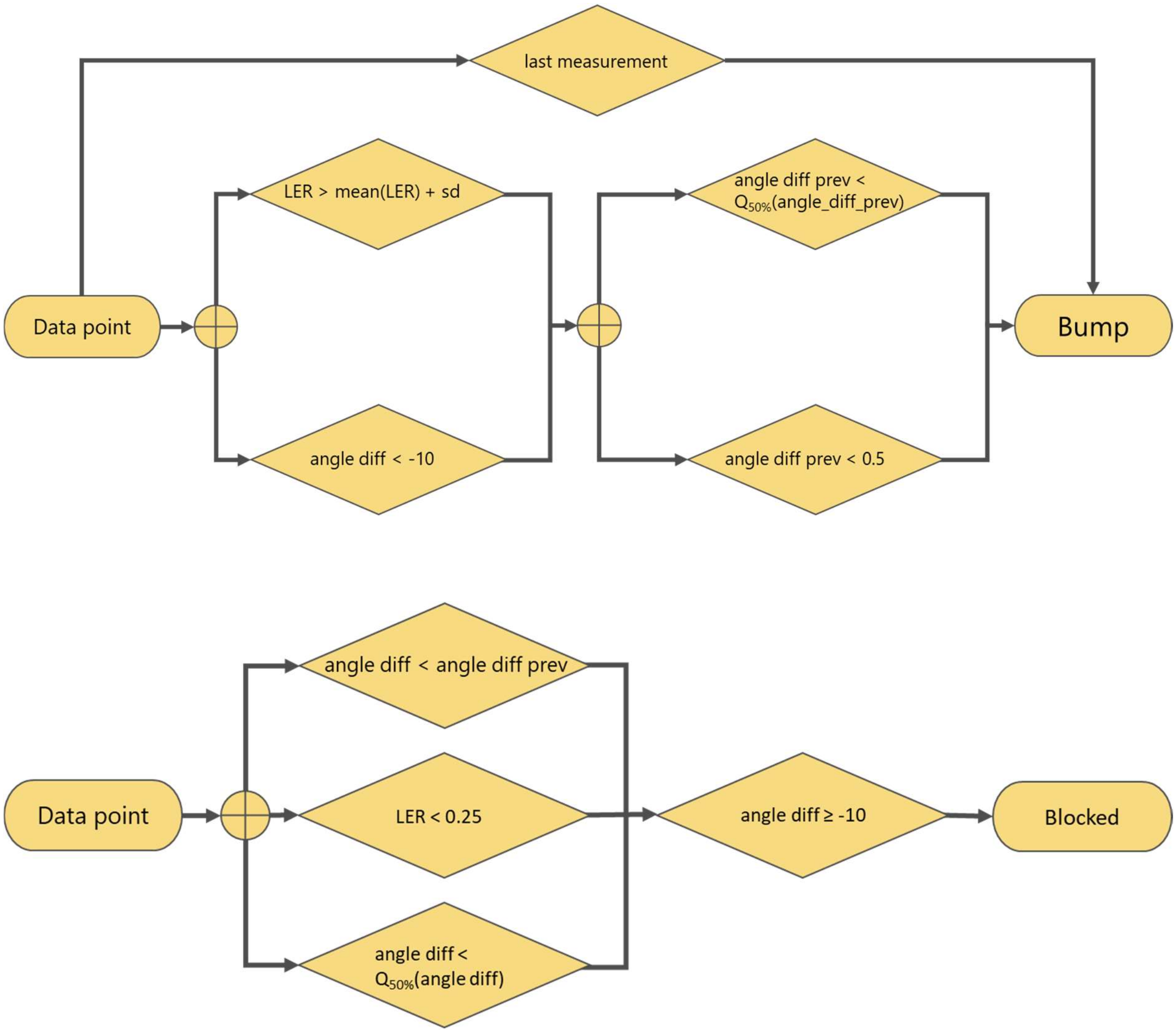


Fig. 15: Flowchart of the procedure to detect start and endpoint of the bump. Endpoint was first estimated (upper panel) by searching for large changes in angle difference (diff) with previous (prev) low changes. For each bump, the starting point of the bump was estimated by finding the first point back in time with a relatively high angle difference.

later LER measurement. Missing environmental data with a maximum gap of two hours were gap filled with linear interpolation. Air temperature and relative humidity data were used to calculate the atmospheric VPD for each measurement.

The scale data were demarcated by a rewatering date and gaps filled using a logistic model (Eqn. 13, SSfpl function in R). If the scale data was completely missing, the pot weight was approximated by averaging all available scale data in the same time range. The starting weight was estimated using the same method, as it turned out to be more stable than single scale values or the mean over a short period.

The LER data was biased due to unsmooth rolling of the pulley leading to “bumps” in the magnetic angle data over time (Fig 14). A simple smoothing would not be efficient as it would also bias proper measurements after the bump and might obscure subtle changes in LER due to changing environmental conditions. We developed an algorithm to detect the start and endpoints of bumps and remove all data in between (Fig. 15). Large LER outliers (> mean + 7 × standard deviation (sd)) were also removed. Leaf length during the week was calculated by subtracting all later changes in leaf length from final leaf length at the end of the week. In cases with large data gaps (> 1.5 days) it was not possible to estimate leaf length from final leaf length and these data were removed from the analysis. Finally, all measurements were also evaluated by eye and erroneous measurements, mostly due to sensor malfunctioning, were excluded from the analysis.

The effect of environmental variables on LER was estimated by using three types of response curves: the Johnson equation (Eqn. 7), linear equation (Eqn. 12) and a four-parameter logistic equation (Eqn. 13):

$$y = mx + q \qquad \text{(Eqn. 12)}$$

$$y = A + \frac{B-A}{1+e^{\frac{xmid-x}{scal}}} \qquad \text{(Eqn. 13)}$$

Where y is the response variable, x is the prediction variable, m is the slope, q is the y-axis intercept, A and B are the value obtained at low and high x values, respectively, xmid is the x coordinate of the inflection point and scal is the inverse of the slope at the inflection point. The modelling was done in a step-by-step approach separately for each genotype. The data were reduced to a subset where all environmental conditions were constant except for the one to be modelled. First the effects of temperature, VPD and illuminance were estimated by removing all data affected by soil water deficit (GWC < 0.5) and using only data of the first two days. Leaf growth was expected to be still in the linear phase (Fig. 3a) and therefore not affected by leaf length. Temperature response was modelled according to the Johnson equation with fixed parameter values according to Parent and Tardieu (2012) and genotype specific scaling parameter *a*, whereas for VPD and illuminance a linear response was assumed (chapter 2.1.5). In the second step, the effect of leaf length was modelled by including data twelve hours after rewatering. The resulting model which considered the effect of temperature, VPD, illuminance and leaf length was used to predict LER over the entire week and differences between measurements and model prediction were calculated. The difference was plotted against GWC and fitted with the logistic equation. Outliers were removed or data were replaced with smoothed estimates to ensure convergence in the fitting procedure. Different models with changing number of parameters were compared based on their coefficient of determination (R^2). Parameters and predictor variables with low influence on the R^2 were deleted from the final model.

The procedure with testing multiple models on the same training data set bares the risk of overfitting. Therefore, one measuring week containing 96 varieties was excluded from the fitting procedure and used to validate the final genotype specific model parameters. Additionally, the parameters were compared against a study evaluating the effect of temperature on stem elongation, using the same genotypes (Kronenberg et al., 2017 and newer unpublished data). The response to temperature was expected to be similar between stem and leaf elongation.

Genome-wide association study (GWAS) was performed to identify genomic regions associated with the genotype specific parameters. Significant QTLs were reported and further analysed to find candidate genes which might influence LER response to environmental conditions.

3.8 Statistical software

Experimental design, data preparation and statistical analysis were all done in R (R Core Team, 2017)

4. Results

4.1 Data quality and filtering

We measured in total 485’159 LERs. About 10% of the data were rejected due to the unsmooth rolling of the pulley (Table 3). The detected bumps in the angle curve (Fig. 14) were visually inspected. In general, the performance of automatic data filtering was satisfactory. Bumps were not detected if there was a larger time interval between measurements. Also, bump detection was impaired if the wheel was not completely blocked but turned at a slower rate.

About 32% of all scale values were obtained by gap filling. More than half of them (18%) could be estimated by relatively accurate logistical and linear interpolation. The measuring instruments for atmospheric environmental conditions like temperature, relative humidity and illuminance were much more reliable and provided data about 87% of the time.

4.2 Phenological stage

Several plants started stem elongation and flowering during the six nursery weeks or on the phenotyping platform. All data from stem elongating or flowering plant were removed for further analysis to avoid the influence of the stem elongation rate on our LER measurements.

The early flowering trait was strongly genotyped dependent. Ten varieties flowered in every replicate including five WW varieties (Table 4). SW varieties were 81 times more likely to flower or stem elongate after 6 weeks ($p < 0.001$, with a Fisher’s exact test). However, two spring wheat varieties never flowered, namely H05606 (entry 389) and P05312 (entry 391).

Table 3: Category, absolute and relative number of leaf elongation rate measurements.

Category	Measurements	% total
Total	485’159	100.0%
Filtering		
Unsmooth rolling	50’505	10.4%
Outliers[a]	693	0.1%
Growing stage[b]	15’345	3.2%
Manual filtering	451	0.1%
Missing env. data	7’150	1.5%
Missing leaf length	12’936	2.7%
Total filtered	**87’080**	**18.0%**
Good quality data	398’079	82.0%

[a] Outliers are data points with ± 7sd or negative LER

[b] Plants which were stem elongating or flowering

Table 4: Varieties which were flowering after seven weeks

entry nr	variety name	type[a]	rep[b]	flowering ratio[c]
10	CHAUMONT	WW	4	1
192	LONA	WW	4	1
27	SIMANO	WW	3	1
277	XENOS	WW	3	1
330	DUXFORD	WW	3	1
380	MONSUN	SW	3	1
383	KWS.SCIROCCO	SW	3	1
385	KWS.AURUM	SW	3	1
386	VANEK	SW	3	1
388	BRYZA	SW	3	1
18	VANILNOIR	WW	3	0.67
349	VELOCITY	WW	3	0.67
381	TAIFUN	SW	3	0.67
382	MARIN	SW	3	0.67
384	KWS.CHAMSIN	SW	3	0.67
387	TRAPPE	SW	3	0.67
23	HANSWIN	WW	3	0.33
46	BUTEO	WW	3	0.33
303	RUBENS	WW	3	0.33
393	P06079	SW	3	0.33

[a] WW: Winter wheat, SW: Spring wheat

[b] number of replicates

[c] flowering plants after seven weeks divided by number of replicates

4.3 Final leaf length

Final leaf length was also influenced by the genotype. Most varieties had average leaf lengths between 240 to 300 mm (Fig. 16). The varieties with an average leaf length larger than 350 mm were significantly different from varieties at the lower tail of the distribution ($p < 0.05$). The variety with the longest leaves was BANQUET (entry 278) with an average leaf length of 409 mm.

4.4 Continuous measurements

An example of one measuring week of variety LEIFFER (entry 79) is shown in Fig. 17. The temperature was much higher than planed during the entire experiment. 75% of the time it ranged between 25 and 32°C and never dropped below 15°C even at night. The highest temperature was 49.6°C which might have negative consequences for plant survival. The lethal limit for wheat is 47.5°C according to Porter et al. (1999).

The time course of air humidity was closely correlated with temperature (-0.72, Pearson correlation coefficient). In Fig. 17 we clearly see three humidity peaks in the first three days due to the air humidifier. The greenhouse door was closed during the third peak, which led to a much higher and longer effect. Unfortunately, the door had to remain open most of the time to allow better cooling of the greenhouse. Illuminance was also closely correlated to temperature (0.59 Pearson correlation coefficient).

Changes in pot weight showed a clear diurnal cycle with a higher decrease during the day when VPD was low and plants had a high transpiration rate. Transpiration and evaporation of each pot resulted in a total evapotranspiration rate of about 50 g water per day during the first three days. We found no significant differences in transpiration rate between the varieties. The problem was an unstable emergence rate which resulted in changing number of plants per pot. This led to high variation of transpiration rate between replicates. The transpiration rate levelled off when GWC reached very low values (GWC ≈ 0.5 g/g dry matter). The ongoing transpiration under low GWC conditions can be explained by the high water availability in the substrate. The pF curve showed that water should be plant available up to GWC of 0.386 g/g dry matter, assuming a permanent wilting point of $10^{4.2}$ hPa.

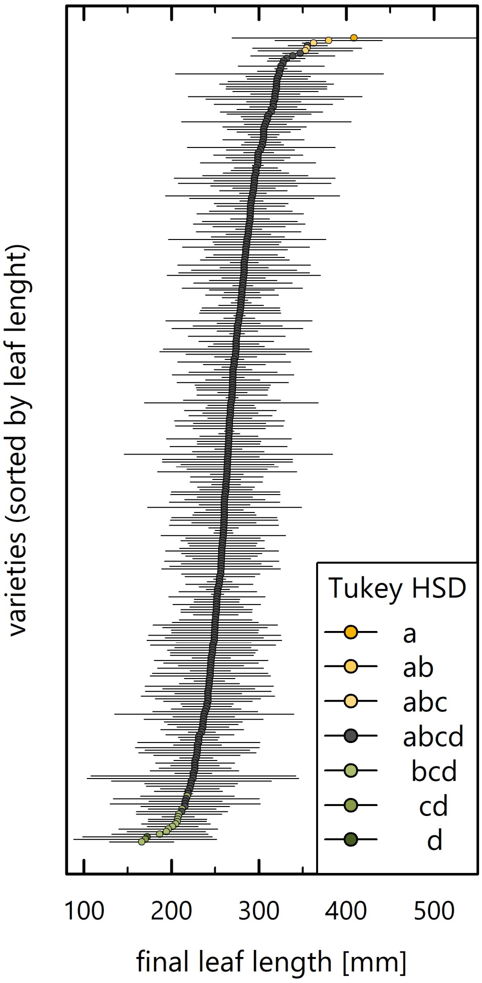


Fig. 16: Final mean length (point) with standart deviation (line) of each variety.

LER measurements after correction for bumps and outliers still scattered widely between successive measurements. The scattering might be related to the sensitivity of the measuring method on vibration and air draft which caused small changes in the magnet angle. Nevertheless, the strong dependency on temperature and GWC was visible, especially by smoothing the temporal course of LER with locally weighted kernel regression (loess function, R).

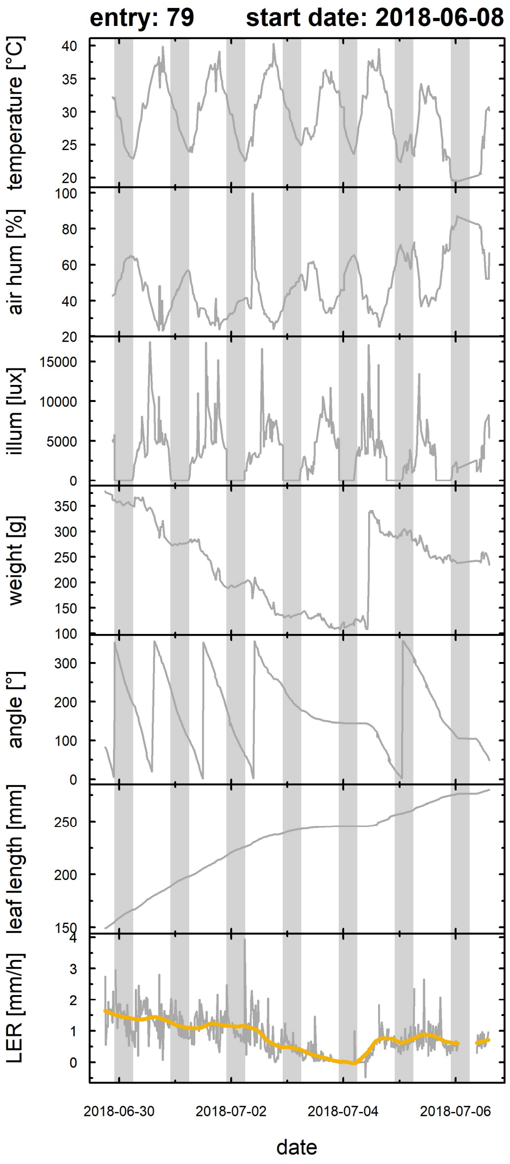


Fig. 17: Time course of measurements in one week for variety LEIFFER. Yellow line in lowest panel shows smoothed leaf elongation rate.

4.5 Model fitting

4.5.1 Temperature

The temperature effect on LER was badly represented by the Johnson equation (Eqn. 7) using parameter values presented in Parent and Tardieu (2012). The peak of LER in our data was 4.8°C higher (32.4°C compared to 27.6°C) and did not decrease as fast at lower and especially at higher temperatures. The strong deviation forced us to adapt the parameters $\Delta H^{\ddagger}_{A}$, ΔS_{D}, ΔH_{D} to get a better estimate of the normalized Johnson equation. For this purpose, LERs were normalized to the rate at 20°C separately for each genotype and the parameters were estimated using nonlinear least square estimation (nls, R). The correction of the parameters led to a clear increase in R^2 of our model (Table 5, model 1 compared to model 2). LER corrected for the effect of temperature using the new temperature response curve led to temperature independent LERs over the entire experiment, which was clearly not the case with the original parametrisation. The fitted normalized model was then used to estimate the genotype specific scaling factor *a* (Fig. 18). LER was expressed in a temperature corrected form.

4.5.2 VPD and illuminance

Adding linear effect of VPD and illuminance with an intercept slightly increased model prediction (Table 5, model 3 and 4). However, the increase was rather low considering that we added two explanatory variables and 3 genotype specific parameters. An inspection of the parameters revealed an inconsistent effect of both variables. Many genotypes even had a positive effect of VPD and illuminance on LER. For these reasons both parameters were excluded from the final model.

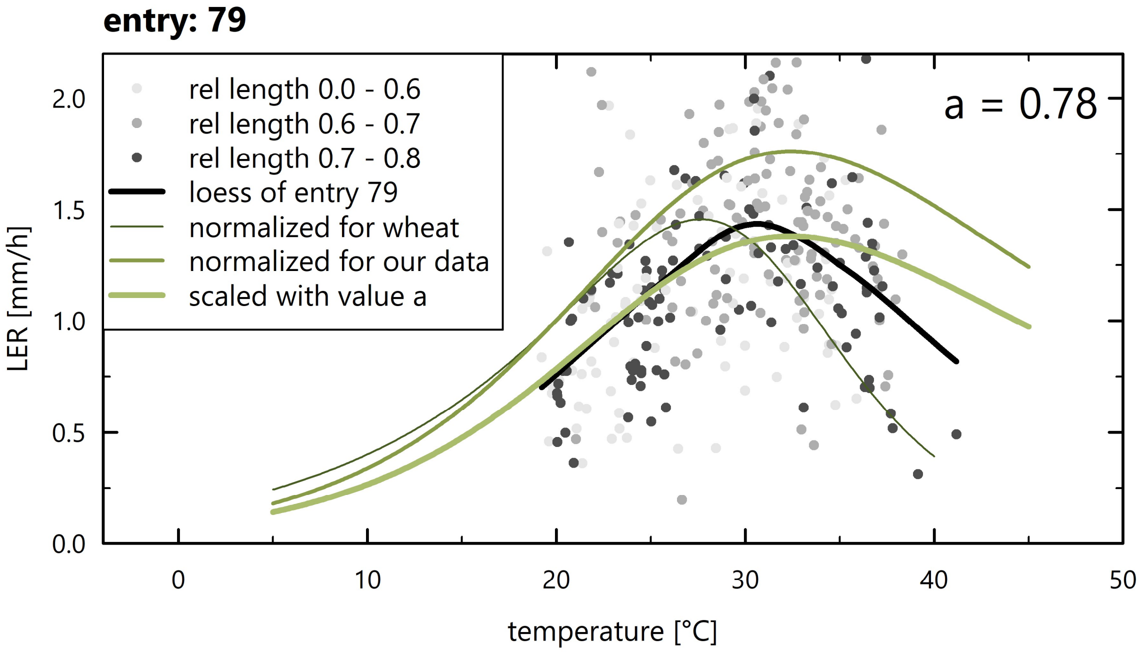


Fig. 18: Temperature response shown for variety LEIFFER. Normalized temperature response curve for wheat (Parent and Tardieu 2012) was shifted to the left compared to the smoothed data (loess). Therefore, a new normalized temperature response curve was fitted to our data. For each variety the scaling factor *a* was determined indicating the absolute height of the data compared to the normalized curve.

4.5.3 Leaf length

Leaf length had a clearly negative effect on growth in all varieties. R^2 was substantially increased by using relative leaf length, obtained by dividing leaf length with final leaf length (Table 5, model 5 and 6).

4.5.4 GWC

The visual inspection of the LER over time already shows the strong effect of soil water deficit on LER (Fig. 17). This effect was also clearly visible by plotting differences between measured LER and predicted LER based on model 6 (Table 5) against GWC (Fig. 19 left). However, we still had some difficulties fitting a logistic curve due to variation in the linear part at high GWC. LER data at GWC > 2 or even GWC > 1.5 needed to be excluded to ensure convergence of the logistic parameters in some varieties. Some parameters of the logistic model changed considerably by adjusting the upper GWC filter. Therefore, we decided to replace the four parameters of the logistic model with parameters which are more stable on changing GWC filters and more easy to interpret. We used GWC_{high} describing the GWC level at which LER starts decreasing (5% decrease) and GWC_{low} representing the GWC level at which LER completely stops. The first was determined based on the logistic model but was clearly more stable compared to other parameters in the model like xmid or scal (Eqn. 13). The second was determined by looking for the upper limit GWC were LER was close to zero (Fig. 19). The new parameters were included in a piecewise linear (PL) function serving as a scaling factor for the LER model:

$$PL = 1 \quad for\ GWC > GWC_{high}$$

$$PL = 0 \quad for\ GWC < GWC_{low} \qquad \text{(Eqn. 14)}$$

$$PL = \frac{GWC - G_{high}}{GWC_{low} - GWC_{high}} - 1$$

$$for\ GWC_{low} < GWC < GWC_{high}$$

Predictions based on the PL function were similar to those of the logistic model

Table 5: Model performance in predicting leaf elongation rate

Model	Predictor variables	Overall parameters^A	Genotype specific parameter	Model	Data used for model fit	$R^{2,\ B}$
1	T	$\Delta H^{\ddagger}_{A,}$ ΔS_D, ΔH_D		LER = *JoEqn*(T)	GWC > 0.5, before rewatering	-0.13
2	T	$\Delta H^{\ddagger}_{A,}$ ΔS_D, ΔH_D		LER = *JoEqnFit*(T)	GWC > 0.5, before rewatering	-0.05
3	T	$\Delta H^{\ddagger}_{A,}$ ΔS_D, ΔH_D	a	LER = *JoEqnFit*(T, a)	GWC > 0.5, before rewatering	0.08
4	T, VPD, Illu	$\Delta H^{\ddagger}_{A,}$ ΔS_D, ΔH_D	i, a, b, d	LER = i + *JoEqnFit*(T, a) + b VPD + d Illu	GWC > 0.5, before rewatering	0.11
5	T, AL	$\Delta H^{\ddagger}_{A,}$ ΔS_D, ΔH_D	i, a, e	LER = i + *JoEqnFit*(T, a) + e AL^2	GWC > 0.5	0.27
6	T, RL	$\Delta H^{\ddagger}_{A,}$ ΔS_D, ΔH_D	i, a, e	LER = i + *JoEqnFit*(T, a) + e RL^2	GWC > 0.5	0.35
7	T, RL, GWC	$\Delta H^{\ddagger}_{A,}$ ΔS_D, ΔH_D, A, B, xmid, scal	i, a, e	LER = i + *JoEqnFit*(T, a) + e RL^2 + *FPL*(GWC)	All	0.29
8	T, RL, GWC	$\Delta H^{\ddagger}_{A,}$ ΔS_D, ΔH_D	i, a, e, A, B, xmid, scal	LER = i + *JoEqnFit*(T, a) + e RL + *FPL*(GWC, A, B, xmid, scal)	All	0.44
9	T, RL, GWC	$\Delta H^{\ddagger}_{A,}$ ΔS_D, ΔH_D	i, a, e, GWC_{high}, GWC_{low}	LER = (i + *JoEqnFit*(T, a) + e RL) × *PL*(GWC, GWC_{high}, GWC_{low})	All	0.43

T = temperature, VPD = vapor pressure deficit, Illu = illuminance, AL = absolut leaf length, RL = relative leaf length, GWC = gravimetric water content, $\Delta H^{\ddagger}_{A,}$ ΔS_D, ΔH_D parameters of Johnson equation (see Eqn. 7), A, B, xmid and scal parameters of the four parameter logistic curve (Eqn. 13), i = intercept, a = temperature response scaling factor, b = slope of VPD response, d = slope of Illumination response, e = slope of the squared leaf length response, GWC_{high} gravimetric water content where leaf elongation rate starts decreasing, GWC_{low} gravimetric water content where leaf elongation completely stops, JoEqn = Johnson equation (Eqn. 7), JoEqnFit = Johnson equation with adapted parameters, *FPL* = four parametric logistic equation, *PL* = piecewise linear equation.

[A] Parameters equal for all genotypes.

[B] Coefficient of determination in predicting all data. Negative values were caused by models which were only fitted on a subset.

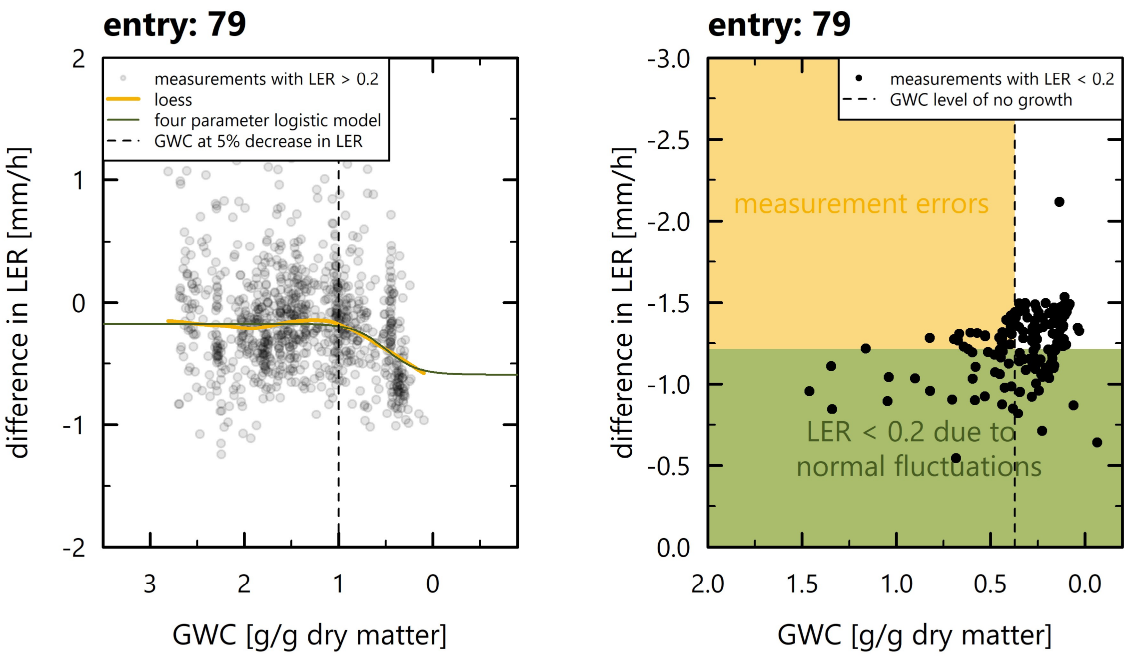


Fig. 19: Determination of GWC_{high} and GWC_{low} in variety LEIFFER. Difference in LER referes to calculated difference between modeled LER based on model 6 and measured LER. Left panel: GWC_{high} represents the startpoint in decreasing LER. It was determined by fitting a logistic curve to LER response to GWC excluding LER values close to zero. GWC_{high} was located at 5% decrease of the logistic curve. Right panel: GWClow represents the GWC level at which leafes completely stops elongating. It was estimated by first excluding all values which had a low LER prediction based on model 6 (green area). The upper GWC limit of the remaining values was then estimated by using the 20% quantile, to exclude outliers from influencing the parameter estimation.

Table 6: Summary statistics of the major parameters used in model 8 and 9

parameter	observations	mean	median	min	max	sd	CV[a]
a	317	0.842	0.845	0.447	1.368	0.149	0.177
i	317	0.602	0.596	-0.120	1.286	0.190	0.316
e	317	-1.282	-1.302	-2.572	0.400	0.394	-0.307
GWC_{high}	317	1.136	1.080	0.100	3.150	0.444	0.391
GWC_{low}	313	0.352	0.325	-0.056	1.416	0.219	0.622
A	309	-1.102	-1.088	-2.899	-0.115	0.434	-0.394
B	309	-0.001	-0.007	-0.451	1.083	0.112	-111.964
xmid	309	0.384	0.381	-0.244	1.443	0.174	0.453
scal	309	0.352	0.325	0.115	1.821	0.144	0.408
Final length	316	268	266	166	409	32	0.125

[a] Coefficient of variation

(Table 5, model 8 and 9) but reduced the number of parameters by two.

4.5.5 LER Prediction

The models containing temperature, relative leaf length and GWC were able to accurately predict the diurnal and weekly course of the LER (Fig. 20). R^2 of the models are still seemingly low (0.44). However, it should be considered that the measured LER scattered widely between successive measurements which is probalby attributed to the precision of the measuring method. The measurement noise can be reduced by smoothing LER time courses or aggregating over longer time periods. Both methods led to $R^2 > 0.7$.

4.6 Model parameters

Summary statistics for the final model parameters are shown in Table 6. Most parameters deviated significantly from normal distribution ($p < 0.05$, Shapiro-Wilk-Test) but visual inspection still showed distributions close to normal but slightly skewed or with heavy tails. Parameter *a* which represents the growing rate at 20°C was 0.84 mm/h on average over all varieties. This is comparable to other studies in wheat

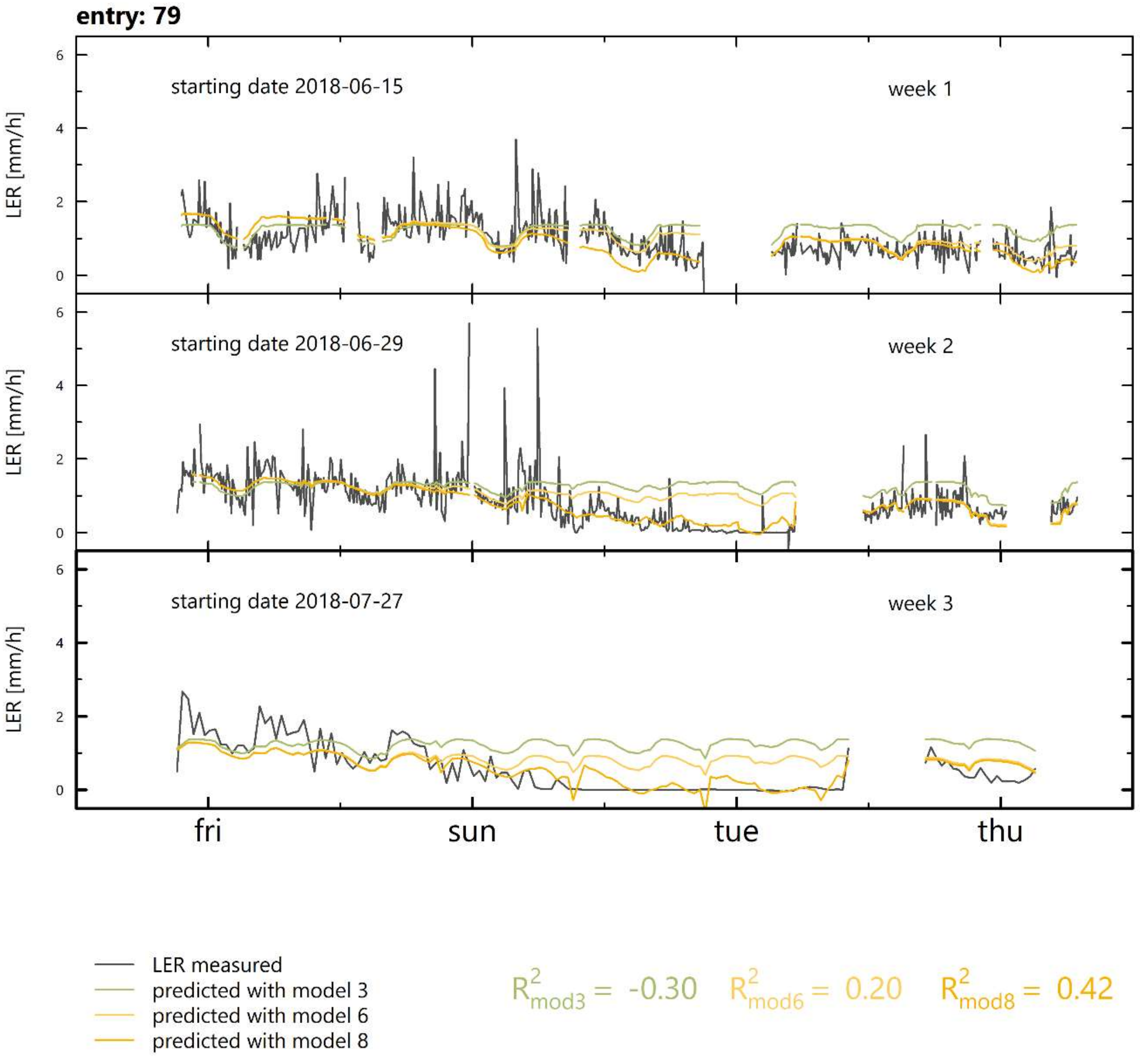


Fig. 20: Model predictions for the three replicate weeks of variety LEIFFER. Model 3 was only fitted on a small subset of the data at the beginning of the week, which explains the high prediction values.

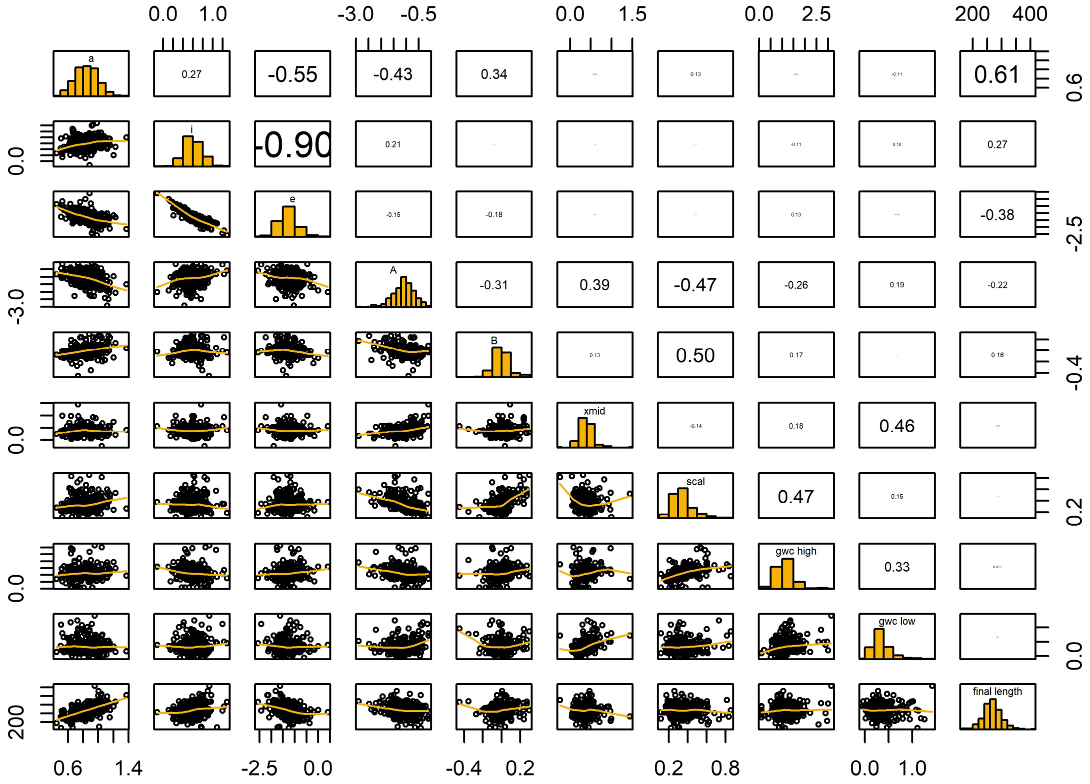


Fig. 21: Correlation table for parameters of model 8 and 9. Fontsize of the correlation number is according to its absolute value.

(Nagelmüller et al., 2016). GWC parameters could be converted to soil water potential using the pF curve (Fig. 12). Therefore, an average genotype would slow down LER at -0.1 MPa and stop growing at -1.9 MPa soil water potential. GWC_{high} is comparable to values found for maize by Reymond et al. (2003) where LER was barely affected at -0.1 MPa. However, in the same study GWC_{low} was estimated between -0.575 to -0.350 MPa which would be far above our values found in wheat.

With regard to differences between WW and SW, SW was found to have higher *i* and significantly reduced *e*, although not significant (p = 0.11 for *i* and p = 0.17 for *e* using a Student's t-test). All other parameters did not differ between wheat type, registration year or registration country.

The correlation table between the parameters revealed some interesting relationships (Fig. 21). Parameter *a* was negatively correlated with *e* and positively with final length. A genotype with a high base growth rate under good growing conditions, expressed by a high parameter *a*, had a steeper decrease in LER towards final leaf length. If it is assumed that LED is relatively fixed in this population then, (as evidence by all genotypes being at similar leaf numbers), then genotypes with higher *a* must have a higher *e* to arrest leaf growth. The link of *a* with final leaf length supports the hypothesis in chapter 2.1.4 that higher LER finally results in longer leaves as it is independent from LED. Morover, the

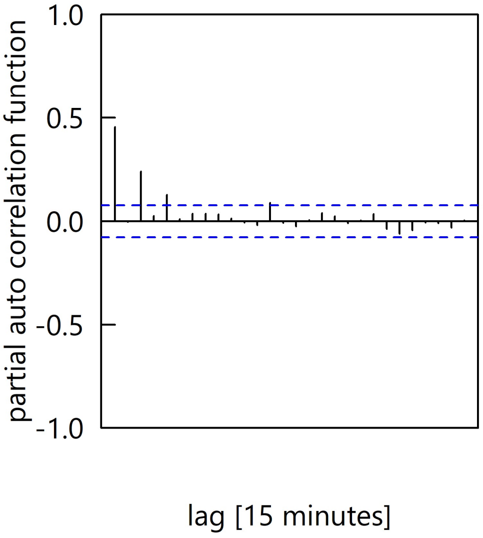


Fig. 22: Partial auto correlation function of regularly spaced measurements obtaind with linear interpolation.

parameter *i* and *e* are strongly negatively correlated. This might indicate that leaf growth was already decreasing due to leaf length in the first three days, from which scaling factor *a* was estimated. A strong negative effect of leaf length on parameter *a* resulted in higher intercept *i* which compensated for higher growth rates at the beginning of the week when the leaf was still short. The correlation table also shows a positive correlation between xmid and GWC$_{low}$ as well as between scal and GWC$_{high}$. A flat logistic curve with a small slope at the inflection point (high scal) shows that the decrease in LER already started at much higher GWC values. The position of the inflection point (xmid) was closer to GWC$_{low}$ and explains the correlation between these parameters.

4.7 Model validation

We report by intension no model fit *p*-values as the data showed high temporal autocorrelation between the residuals of the model (Fig. 22). On a theoretical basis, temporal autocorrelation biases standard error estimates of parameters but not the parameter estimates itself. Thus, it is absolutely legitimate to estimate parameters from data which are autocorrelated over a relatively short period compared to the entire measurement duration.

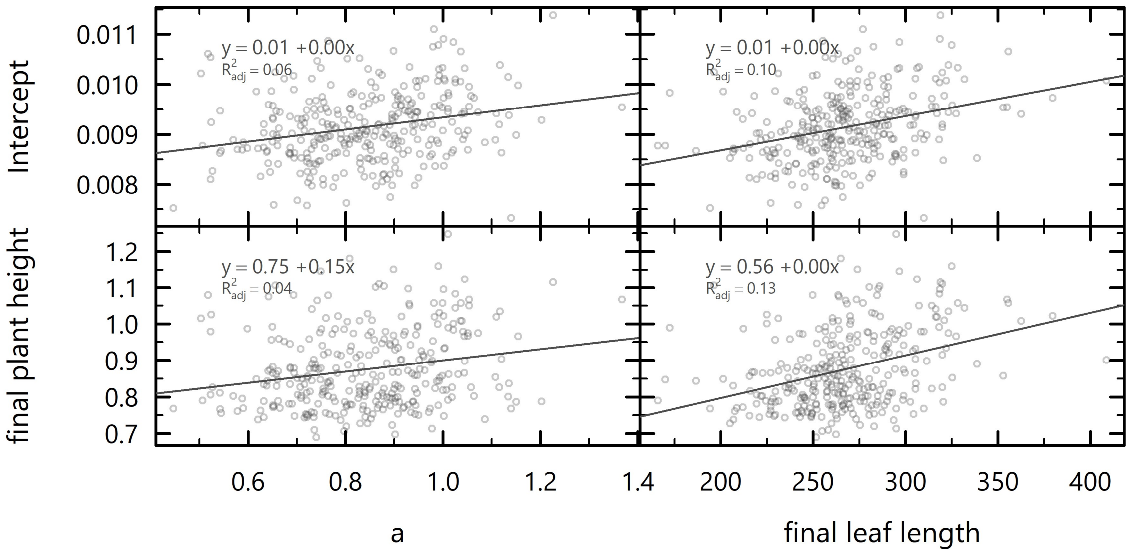


Fig. 23: Linear regression between our traits (*a* and final leaf length) and comparable traits of a unpublished study about stem elongation. Intercept referes to the intercept of stem elongation response to temperature.

The model was therefore only validated by testing it on a data set which was not used for model fitting containing 64 varieties. R^2 of the fitting was 0.40 and therefore almost as high as the model fit using the training set. However, it should also be mentioned that there was a large variation in R^2 between different varieties. Some had R^2 values higher than 0.7 whereas others even had negative values. The time course of LER with a negative R^2 in the model fit was visually analysed. They often revealed a very untypical growth pattern with sometimes heavily decreased base growth rate at the start of the week. This might be attributed to some other factors influencing leaf elongation rate that are not included in our model, for example pest and diseases.

The correlation between parameters of our study and a study analysing stem elongation were quite low. However, we found a small correlation between our scaling parameter *a* and the intercept of the temperature response in the stem elongation study (Fig. 23). Both parameter lead to an increase of the temperature response curve. Greater, positive, correlations were found between final leaf length and final plant height (0.36 Pearson correlation coefficient).

4.8 Candidate genes

The GWAS found one significant association between a single nucleotide polymorphism (SNP) marker and GWC_{low} but no association with GWC_{high} ($-\log_{10}(p) > 5$). The SNP was located on the 4A chromosome. In close proximity to the SNP, several interesting candidate genes were identified which need to be further investigated.

5. Discussion

This study tested a fast screening method to phenotype the drought tolerance of 320 wheat varieties. We used 96 devices measuring LER and used the measurements to develop a model which was able to predict LER with an R^2 of 0.44. The main factors affecting LER were temperature, relative leaf length and GWC.

5.1 Phenotyping platform

The MEU phenotyping platform was able to measure LER and environmental data on a high temporal frequency. Most of the measurement devices worked reliably and provided highly accurate measurements. However, data quality could be improved by fixing two issues. First, contained large data gaps which were caused by regular disconnection of USB devices. The data gaps caused problems in the data analysis and hampered the possibility to consider the temporal autocorrelation in the statistical analysis. Secondly and more important, the unsmooth rolling of the pully led to large outliers which needed to be filtered out and it increased the experimental error of all LER measurements. An improvement in this issue would not only lead to more regular measurements but should also increase model predictions. A redesign of the ball bearing part should be considered. Although this factor was most evident at low rotation speeds.

5.2 LER Modelling

A surprising finding during model fitting was the strong deviation of temperature response from the Johnson equation with parameter values reported in Parent and Tardieu (2012). The deviation was probably caused by different temperature measuring methods used in the two studies. In Parent and Tardieu (2012), temperature was measured as meristem temperature whereas we used air temperature. The buffering effect of soil and leaf sheath as well as the cooling effect of transpiration would well explain the shift in peak temperature and the flatter response in more extreme temperature regions (Fig. 18). Another aspect which was not considered by Parent and Tardieu (2012) is phenotypic plasticity. Plants are able to adapt their temperature response based on long term conditions they are exposed to (Atkin et al., 2006). The plants in our study were facing high temperature in the plant nursery and might have adapted their temperature response to those conditions. A wider range of temperature, especially in the lower temperature range (0 to 15°C) would be preferable in order to improve curve fitting for temperature response. The growth response in this temperature region is also more relevant for European climate conditions. Moreover, in the work by Parent and Tardieu (2012), they considered 8 genotypes representative of the wheat species. Whilst, on average, we observed a similar trend, exceptions did occur and genotypes were found which showed no decrease in *a* at higher temperatures.

VPD was expected to have a strong negative effect on LER. However, the effect was excluded from the final model without having a strong negative effect on R^2. This fact should not be interpreted in a way that VPD had no effect on LER in our experiment. The high correlation between VPD and temperature made it impossible to statistically disentangle the two effects. The parameters of the Johnson equation therefore contain mixed information about LER response to temperature and VPD. The air humidification would need to take place over a longer period (e.g. an entire day) to

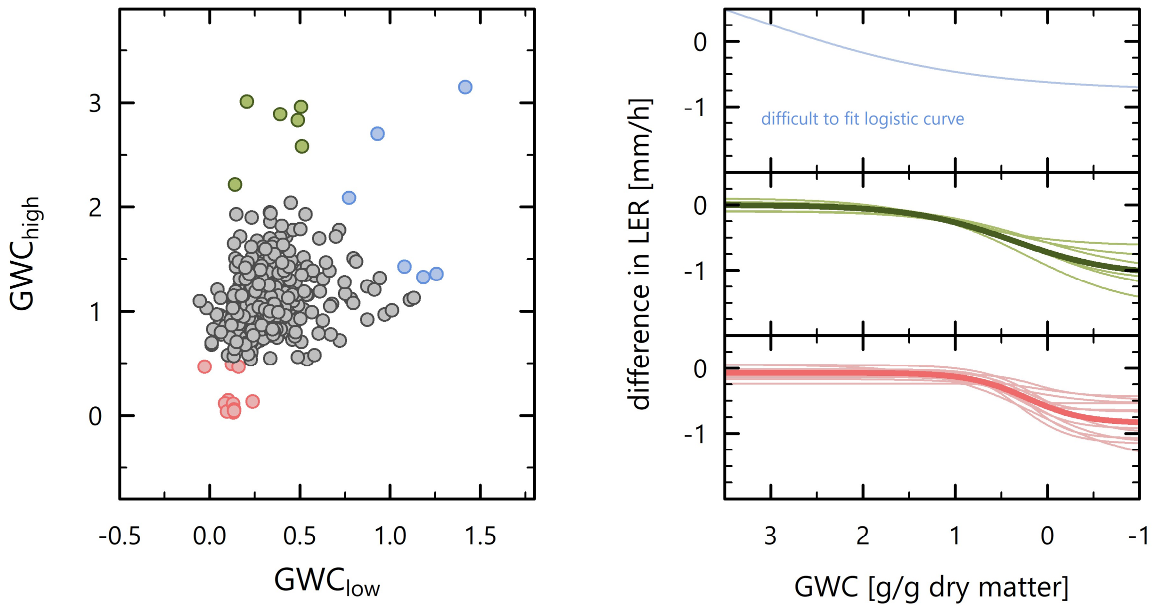


Fig. 24: Grouping varieties according to their parameters describing the response of LER to GWC. Difference in LER referes to calculated difference between modeled LER based on model 6 and measured LER. Drought susceptible varieties (blue) show a immediate decrease in LER if GWC decreases. Varieties with a continuous response (green) show also a decrease in LER at high level but decrease at a much lower rate. Drought tolerant varieties (red) do not react to GWC until they can no longer take up water from the soil.

separate the two processes and get estimates for the response curve of both.

Predictions based on model 8 and 9 were still far from perfect. This was probably caused by the large experimental noise which could be reduced by aggregating measurements over a longer time period. However, in order to detect the short-term responses to rapidly changing environmental factors like light and temperature, data with high temporal resolution would be preferred. Still, model predictions were valid for the testing set. This indicates a good parameter estimation after only three weeks despite of experimental noise and associated low R^2 values. One other aspect to consider is that the model is not representative for all genotypes. Critically, modelling response to GWC assumes a plant responds to a given environmental factor. However upon manual inspection of the results, genotypes were observed which did not respond to GWC. This is not to say that some genotypes can grow without water, but they are insensitive to decreases in water availability. Previous work by the MPB group uncovered such responses in *Lolium perenne* genotypes. In collaboration with other groups they found that such genotypes typically perished in the field, presumably on account of their inability to regulate and conserve water in dry periods. However, in wheat the inverse might be more promising, as wheat is an annual plant (unlike *L. perenne*) and completes its life cycle before dry periods are more prevalent. Therefore, future studies of such genotypes might be fortuitous for wheat breeding in Europe.

5.3 Traits

Determination of final leaf length and early flowering were not the main aim of this study. However, both were found to be genotype specific. Early flowering was especially heritable which is also supported by literature (Langer et al., 2014). Ten WW varieties had at least one replicate flowering after only seven weeks without vernalisation. They might be interesting candidates for speed breeding approaches.

The drought tolerance of a variety can be characterized by GWC_{high} and GWC_{low}. We identified three extreme groups of varieties by combining information of the two parameters (Fig. 24). The drought susceptible plants show a high GWC_{high} as well as a high GWC_{low}. They immediately stop growing when sensing the first signs of a drought. Therefore, they might be best adapted to a very pronounced drought scenario which severely threatens the survival of the plant. In the trade-off between photosynthesis and transpiration, they favour to reduce their transpiration even if it reduces the potential amount of intercepted light for photosynthesis, from decreased leaf gowth. The second group contains of varieties, which already decrease their growth at very high GWCs but continue growing at lower rates even at a severe soil water deficit. Those varieties are highly adaptive over a large range of GWC and might be good candidates for studying processes involved in LER regulation and signal transfer from roots to shoot. Finally, the group of the drought tolerant varieties, showing almost no response of LER to GWC up to a level where growth is probably simply impossible due to decrease in turgor pressure. Such a strategy is fatal in a severe drought event, which is why these varieties are best suited for regions with very low risk for sever terminal drought (Tardieu, 2012). The majority of the varieties showed similar response with GWC_{high} and GWC_{low} 1.1 and 0.6, respectively. Similar GWC values for the large majority of the GABI wheat varieties is expected as all European varieties are adapted to a similar rather humid environments, otherwise they would have been selected against. A larger variance between genotypes would be expected by comparing the GABI what panel with plants adapted to an environment with regular severe droughts as it is known in Australia or the middle east (Zhang et al., 2015).

Grouping the varieties by their country of registration did not reveal any pattern in the distribution of any parameter. Also, we observed no temporal trend in a parameter value. 50 years of wheat breeding in Europe led not to a shift in LER response to environmental conditions. This show that the responses of varieties seem to be well optimized to the environmental conditions of recent years.

The tendency for higher *i* values and lower *e* values in SW shows that SW varieties seem to have a faster leaf cycle (phyllochron) with higher growth rates at low relative leaf length and a steeper decrease when reaching final leaf length. The same results were also obtained by McMaster and Willhelm (1995). The result is intuitive, as SW has to complete its growing cycle in a shorter period of time.

5.5 Application

The MEU phenotyping platform as shown in Fig. 9 has a wide range of possible applications in breeding, genomic research, physiology research and agronomy. The main intention of this study was to test the MEU as an early breeding stage phenotyping tool to select for drought tolerance. The platform was found to have fulfilled at least three of four criteria required for an efficient phenotyping of drought tolerance as mentioned in chapter 1: (i) The method was fast. Accurate estimates of model parameters were obtained within three weeks. (ii) The method was highly automated. It took some time get the system running but afterwards the main task was only to provide new plant material from the nursery. (iii) The method was reproducible as shown by R^2 in predicting the validation set. The main question remaining concerns yield. In maize, LER response to drought were successfully implemented in crop models and improved yield prediction (Chenu et al., 2008). Unfortunately, we have currently no yield data available for the GABI wheat panel. A valuable source for yield data at least for the Swiss varieties would be the variety trials run by Agroscope (Swiss Confederation's centre for agricultural research). Reliable yield data will be inevitable to incorporate LER response parameter into existing crop models to predict yield in a certain drought scenario. Assuming a similar crop model accuracy as in Chenu et al. (2008), estimates of LER response parameters would be a valuable selection criterion for breeding on high yield under drought conditions.

Another field of application are association studies, which try to find genomic regions influencing drought tolerance, and functional genomics studies, seeking to understand the molecular mechanisms of drought tolerance. A better understanding of the molecular basis might finally facilitate breeding through marker associated selection or genomic prediction.

The platform offers great opportunities to study LER response to a wide variety of environmental and biotic factors like CO_2, air pollutants, nutrients, soil properties, salt stress, pests and diseases. In this study extraneous factors were controlled as much as possible. However, there was large infestation with thrips (*Haplothrips tritici*) during week 9 to 11 (20.07.2018 – 09.08.2018). It was difficult to say how large the effect of the thrips was, as we observed in general a relatively large between week variation (significant effect with *p*-values between 0.09 to < 0.001 but without considering temporal autocorrelation, ANOVA). LERs were generally a bit lower towards the end of the experiment.

MEU has some clear advantages over existing LER tracking system (Nagelmüller et al., 2016; Sadok et al., 2007b). Rotating displacement transducers as used in the Montpellier plant phenotyping plantform (Sadok et al., 2007b) are relatively expensive, which would hamper a wide application in applied breeding. Imaging-based marker tracking as used in Nagelmüller et al. (2016) comes with a high workload (image analysis), requires a linear plant arrangement and is susceptible to small camera movements. At the moment the MEU system is intended for a greenhouse. However, the LER measurement devices should also be ready for the field by adding a battery and a datalogger shield.

6. Conclusions

Advances in sensor technology and digitalisation are highly promising for an application in plant breeding. They allow an automated and high throughput phenotyping of complex traits which would remain unrecognized with classical methods relying on the breeder’s eye. LER response to environmental condition is a trait which largely determine the performance of a genotype within an environment and can be used in crop modelling to estimate yield under a certain drought scenario. Until recently, measuring the trait on a high temporal resolution was extremely laborious and impossible to execute on the large number of new genotypes in a breeding program. In this project, we show a method which could easily be implemented at a low cost. The results of the LER phenotyping combined with envirotyping led to a characterization of a varieties drought tolerance in only three weeks. We conclude that breeding on drought tolerance can be substantially accelerated by applying modern sensor technology in plant breeding.

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Fig. 25: Surprize in the nursery

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Eigenständigkeitserklärung

Die unterzeichnete Eigenständigkeitserklärung ist Bestandteil jeder während des Studiums verfassten Semester-, Bachelor- und Master-Arbeit oder anderen Abschlussarbeit (auch der jeweils elektronischen Version).

Die Dozentinnen und Dozenten können auch für andere bei ihnen verfasste schriftliche Arbeiten eine Eigenständigkeitserklärung verlangen.

Ich bestätige, die vorliegende Arbeit selbständig und in eigenen Worten verfasst zu haben. Davon ausgenommen sind sprachliche und inhaltliche Korrekturvorschläge durch die Betreuer und Betreuerinnen der Arbeit.

Titel der Arbeit (in Druckschrift):

Genetic determinants of leaf growth response to environment in wheat

Verfasst von (in Druckschrift):
Bei Gruppenarbeiten sind die Namen aller Verfasserinnen und Verfasser erforderlich.

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ETH zürich

Molecular Plant Breeding

Bachelor/Master thesis project

Precision farming of wheat for future environments

Background

Wheat (*Triticum aestivum* L.) is a major cereal crop, annually grown on more than 200 million hectares worldwide. The demand for wheat is expected to increase, however, the current gains in productivity are around half of that required (Rosengrant & Agcoili, 2010; Reynolds et al., 2012). In the future, adverse environmental conditions are a major threat to wheat production. However, little is known about which genetic loci control growth with respect to environmental factors. The MPB group is currently using a phenotyping platform to determine the plant response to multiple environmental traits including temperature, humidity and soil water availability. This project seeks to apply this exciting technology to improve wheat breeding for the future.

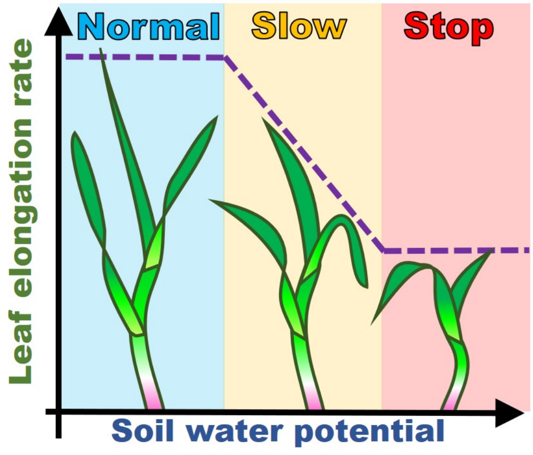
Objectives

The aims of this project are:

- Phenotyping leaf growth with respect to environmental influences in wheat
- Identify genetic loci controlling leaf growth

Research approach

This project will make use of a new phenotyping platform developed at ETH Zurich to record environment and leaf growth at high precision and frequency. This will be used with the GABI wheat panel, which consists of more than 300 wheat genotypes, representing commercial wheat lines used in Europe over the last 50 years. Together, the phenotypic data and existing genetic data will be combined to identify genetic loci controlling these traits.



You will learn

Methods to describe leaf growth, multi-modelling of traits, quantitative genetics and genome-wide association studies

We are looking for

A motivated student with interest in learning advanced techniques for plant environment phenotyping. No prior experience is required as full training will be provided. This project will start from February 2018 onwards and is suitable for Masters of Bachelor students

Contact

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