

PROJECT FINAL REPORT

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Summary

DROPS has developed novel methods and genetic strategies for a better understanding of yield control under water deficit, based on the premise that any so-called trait/gene of drought tolerance can have positive, negative or no effect depending on the considered drought scenario. It involved public research groups in Europe and Australia who are leaders of their disciplines (physiology, modelling, genetics, statistics, and breeding) and four seed companies who are transferring novel understanding into breeding programs. The project has worked on four target traits, namely grain abortion and vegetative growth maintenance in dry conditions, root system architecture and transpiration efficiency. It dealt with maize, bread wheat and durum wheat. DROPS has:

- Shown that plant hydraulics has a large contribution to the genetic variations of leaf growth and abortion under water deficit, while metabolic aspects had a lower impact than expected. We have developed knowledge-based methods for identifying physiological mechanisms that influence the genetic variability of the four target traits under drought and designed approaches based on new phenotyping techniques. This has resulted in the identification of novel mechanisms and in the prediction of traits under diverse environmental scenarios via simulation models. These approaches are based on experiments in a series of trait-specific phenotyping platforms in controlled condition, omic platforms and a network of field experiments across Europe
- Shown that genomic regions controlling yield and the four target traits differ between diverse drought scenarios identified over Europe. We aimed at (i) exploring the natural genetic variability to identify genomic regions controlling yield and traits, (ii) linking trait variations to physiological pathways, genes or genomic regions, (iii) modelling the effects of the allelic diversity on the responses to environmental conditions of the four target traits. Panels of lines (durum and bread wheat) or hybrids (maize) have been analysed in phenotyping platforms and in a network of fields for each species. Association genetics was based on a dense genotyping (e.g. 350,000 SNP markers in maize) and involved phenotypic data in fields and platforms, including variables determined by model-assisted phenotyping.
- Supported crop improvement strategies by estimating the comparative advantages of relevant combinations of alleles under contrasting drought scenarios in Europe. This has been performed by (i) developing a comprehensive and generic crop model that incorporates novel understanding of the physiology and genetics of the four target traits and allows prediction of allelic effects on crop growth, yield and water-use efficiency, (ii) simulating yields of genotypes that differ by traits or alleles, over the variability of drought scenarios occurring across Europe.

A dedicated information system secures and gives access to the whole DROPS dataset. It consists of a centralized database for phenotypic data collected in field experiments, and of web services that connect databases for genotypic, omic and phenotypic data collected in platforms. The information system includes tools for assessing the validity of phenotypic measurements and for relating them with environmental variables. This allows joint analysis of (i) all phenotypic data (field and platforms) together to environmental conditions, and (ii) genotypic data for genetic analyses, essentially association genetics.

Results have been disseminated via (i) the participation of four seed companies to DROPS, who are using methods and alleles developed by the consortium, (ii) via a partnership with the breeder association EUCARPIA with whom we have held a common final conference, and

(iii) via hands-on courses and virtual courses through the website, aimed at presenting methods and approaches for dealing with water deficit.

Context, objectives and strategy

Context

European agriculture is facing declining water availability and a reduction of arable land. Predictions of climate change indicate an increased variability of rainfall in the next 40 years and an increased risk of high temperature and of water shortage during summers. Meanwhile, water for irrigation will be at best maintained at current levels though at higher cost. It is accepted that these events will jeopardize global food security unless large investments are made. New genotypes are therefore needed to at least maintain levels of productivity with reduced water input. This is a challenge for the society, but also for the competitiveness of European seed industry.

During the last decade, most academic effort has been devoted to the identification of single genes that would confer drought tolerance, *via* mechanisms as different as overproduction of growth regulators, tolerance to oxidative stress, changes in metabolic pathways or manipulation of transcription factors. This has resulted in spectacular results in specific, usually controlled, conditions. Approaches based on the exploration of the natural genetic variation of key productivity traits have been more efficient, but the pace of genetic progress must now be accelerated for alleviating four bottlenecks.

- Scenarios of water deficit are diverse in terms of intensity and timing, and are most often correlated with scenarios of high temperature or evaporative demand. It is therefore necessary to dissect this complexity by precisely measuring environmental conditions in field experiments, and by disentangling complex scenarios in phenotyping platforms under controlled conditions.
- While genotyping can now be carried out on thousands of plants with high definition (thousands to millions of markers over the genome), phenotypic analysis at this throughput requires methodological improvement if one wishes to keep its relevance.
- Novel genetic approaches such as association genetics have essentially dealt with simple and highly heritable phenotypic traits such as flowering time or simple metabolic pathways. Using this approach for a complex trait such as drought tolerance is a major challenge, which requires the development of a new phenotyping strategy.
- The agronomic value of an allele or trait in a droughted field varies with the drought scenario. Even in most successful field analyses, carried out across a broad range of water availability, given alleles of quantitative trait loci (QTL) for grain yield usually result in a positive effect in only half of the tested environments and can even become negative in other environments. Hence, even large networks of field experiments are not sufficient to represent the diversity of scenarios in which each allele is tested.

Objective

DROPS objective was to develop knowledge on physiological and genetic mechanisms associated with improved plant performance in a diversity of climatic scenarios involving drought and high evaporative demand (largely linked to high temperature). We aimed at (i) the identification of alleles that affect traits of interest and their effects on yield, (ii) novel mechanisms linking environmental conditions and trait values, and (iii) models able to

simulate the performance of plants carrying these alleles in European scenarios. This knowledge was intended to result in methods aimed at increasing the efficiency of the breeding of genotypes offering high yield under water deficit, enhanced water-use efficiency, or better adaptation to water deficit than current varieties. Four key traits for drought tolerance and water-use efficiency have been studied, namely grain abortion, shoot growth maintenance, root system architecture / efficiency, and transpiration efficiency. This was performed in three species, maize, durum wheat and bread wheat.

State of the art at DROPS submission and progress beyond it

At the time when DROPS was submitted, high throughput phenotyping and genotyping methods were emerging and the use of crop modelling for assessing the effect of genetic variability was at its very beginning. DROPS has had a pioneering role in the use of novel methods and approaches.

- The 'conventional wisdom' in the scientific community was that carbon metabolism is the major player in plant responses to water deficit. However, plants hydraulics was progressing rapidly with the discovery of the roles and control of water channels, with the development of phenotypic methods for characterizing hydraulic conductances of plants and organs, and with methods for high throughput measurements of amounts of transcripts and proteins involved in hydraulics and in metabolism. DROPS has considered both water and carbon-centered views by gathering leading groups associated with each view, who saw DROPS as a way to challenge their approaches. This has had profound consequences on the project, for growth analysis of several organs, for discovery of novel mechanisms and for plant modelling.
- Phenotyping platforms had already been developed and tested for several years by several partners. This was in particular the case for the INRA Montpellier group, who had developed platforms for leaf growth and transpiration, and UC Louvain who had developed a platform for root growth and architecture. Other partners have developed their own platforms, in particular in Adelaide, Bologna and Lancaster.
- High throughput omic platforms had been developed, in particular for high throughput metabolic phenotyping (MPI Golm, INRA Bordeaux, ACPFG Adelaide), hormone measurement (U. Lancaster) and high throughput measurements of transcript abundance (UC Louvain, ACPFG Adelaide).
- Portable weather stations allowing measurement of environmental conditions were available so environmental conditions could be measured in each field and phenotyping platform with a temporal definition of one hour and the sufficient number of technical replicates. It is noteworthy that, at the time when the project was submitted, this was not considered as a high tech strategy. Since then, this approach has been popularized by the "big data" era and the massive investment of Monsanto in 2014, who bought *The Climate Corporation*. Clustering experiments into climatic scenarios and ascribing allelic effects to these scenarios has been announced by Monsanto as a major contribution, but DROPS has proposed this approach as early as 2009. This approach has rapidly diffused in the four seed companies partners of DROPS.
- Conversely, field phenotyping techniques were still in their infancy in 2009. It was therefore not reasonable to propose their use at high throughput, in particular precise measurements of the area and physiological status of leaves of all genotypes via functional imaging using cameras with different wavelengths carried by ground vectors or drones.

Field phenotyping has therefore been based on the measurement of yield components, with innovations such as a reclined field allowing to test which genotypes reach the water table (Bologna), and image analysis of ears of a test genotype in maize.

- High throughput technologies were available for dense genotyping. In 2009, we proposed a genotyping with tens of thousands markers. This number has been increased to hundreds of thousands markers for the three studied species during the course of the project (U. Bologna, INRA Moulon and Adelaide). In maize, which has a rapid decay of linkage disequilibrium with physical distance, this number has now reached the million markers thanks to the exchange of information with a companion project, ANR-PIA Amaizing.
- Mixed models had been developed for analyzing such large datasets by the partners Biometris (Wageningen) and INRA Moulon. However, considerable refinement has been required during the project for taking into account the kinship of diversity panels and for multi-site, multi-trait analyses of the whole dataset.
- Crop models had been developed for most species, especially wheat and maize. However, models were essentially used for simulating the performance of a standard genotype per species, as affected by crop management (i.e. sowing dates, row spacing, or irrigation) or climate change in different regions. Hence, models dealt with "maize" or "wheat" without reference to specific genotypes. A pioneering work had been performed by three partners (U. Queensland, Pioneer-Du Pont and INRA-Montpellier) for simulating the yield of virtual plants characterized by their allelic values at QTLs in a large set of environmental scenarios. DROPS is the first UE project that has developed this approach at a large scale.
- The strategy for data storage and organization was based in most projects on a central repository for the whole project, in which all data were to be centralized. This approach has proved to be inefficient because of the large effort needed for transferring data in the formats of the central repository, and because this repository usually had a short time life after the end of the project. DROPS has innovated by combining a distributed information system for the data collected in genotyping and in each phenotyping platform, and a central database for field data (environmental conditions, meta data and yield components). DROPS approach has been the base for further developments in phenotyping national projects (e.g. FPPN and DPPN in France and Germany), and for the future ESFRI phenotyping infrastructure EMPHASIS, recently accepted for an ESFRI preparatory phase.

Strategy

DROPS strategy was to combine novel approaches presented above as a proof of concept for developing a new strategy for improved plant performance in dry/hot conditions (DROPS definition of "drought tolerance"). For this reason, workpackages have not been distributed by activities (e.g. "genotyping", "phenotyping") nor by species, but attempted to integrate approaches at different levels of organization.

- WP1 has tested methods and models for studying traits under environmental scenarios. (i) We have analysed in detail the mechanisms associated with the genetic variability of each target trait and of its response to water/temperature stresses. We have used for that image analysis and precise sensors, whose outputs were analysed jointly with changes in environmental conditions, transcript levels, metabolites, hormones and water status in studied organs. This has resulted in information on the physiology of studied genotypes, used in further genetic analyses. (ii) We have tested novel sensors to be used in the field and in platforms. (iii) The whole consortium has tested a detailed environmental

characterization in a series of preliminary experiments, in the field and phenotyping platforms. This approach has been massively accepted by public and private partners, and has allowed genetic analysis of the whole dataset taking into account the diversity of climatic scenarios.

- WP2 has explored the natural variation in genetic panels of diversity or mapping populations representing the genetic variability in the three studied species: (i) yield in fields with contrasting climatic scenarios, (ii) the four target traits in phenotyping platforms, and (iii) omic data (eQTLs, QTLs of metabolites or hormones). We have identified genomic regions which control each trait in each platform, and which control flowering time, yield and yield components in each field. Then, datasets were analysed cross sites, traits and scales (omics, detailed traits in platforms, yield components across fields) to identify common genetic controls. This has resulted in a massive dataset allowing one to ascribe the effect of studied alleles to precise phenotypic traits and environmental scenarios.
- WP3 has developed a new-generation crop model for estimating the comparative advantages of relevant alleles and traits in fields with contrasting drought scenarios in European regions. This has needed profound changes in the APSIM model, chosen as the base for modelling, for leaf growth rate, grain abortion and root architecture. Resulting models can use parameters corresponding to each of the hundreds of genotypes, estimated from data collected in phenotyping platforms. The modules corresponding to each trait have been isolated in such a way that they can be implemented in other crop models. This has allowed us to simulate the consequences of relevant alleles on yield in a large number of drought scenarios (25 years in 35 European sites), making it possible to predict response to selection and guide breeding programmes in each region.
- WP4 has organized the dataset and connections between platforms for facilitating the analysis of the whole dataset, with tools dedicated to the collection and visualization of field data, of genotypic data with the connection between markers and genes, and of the physiological role of gene families associated with identified genomic regions. We have reached a consensus for the definitions and units of phenotypic and environmental variable, which is now used in further EU and national projects. Finally, a large effort has been dedicated to develop multi-trait multi-site mixed models allowing analysis of the whole dataset.
- WP5 has disseminated results and methods for assessing and developing genotypes which respond to climatic challenges to European agriculture. This involved a project website, a collection of published papers and the organization of specific courses on phenotyping, association genetics and information technologies. The final scientific DROPS conference, organized with the breeder organization EUCARPIA, has gathered 305 scientists from private and public sectors originating from all continents.
- WP6 has dealt with project coordination, achieved deliverables quality control, has maintained the project website and have migrate it so it can be consulted after the end of the project.

Main results

1. Target trait and water deficit, from analyses to models

The detailed analysis of target traits in phenotyping and omic platforms has resulted in novel views of the control of these traits. This has been formalized into models or concepts for models with short time steps (e.g. hour or minute) considering one organ or one function. Simplified versions of these models have been implemented in the crop model presented in §3.

1.1 Growth Maintenance

1.1.1 A novel approach of temperature effects for the three species: differences in responses between species but not between genotypes within each species.

Temperature fluctuates rapidly and affects all developmental processes. We have proposed a method for modelling temperature-compensated rates, based on the coordination of temperature responses of developmental processes in the range of 6-36°C in several genotypes of maize and wheat (plus 15 species). Germination, cell division, expansive growth rate, leaf initiation and phenology show coordinated responses and follow common laws within each species. The model for response to temperature is based on an equation widely used in ecological studies and in metabolism, but rearranged to apply to the growth response to temperature. The response of each species is characterised by two parameters only.

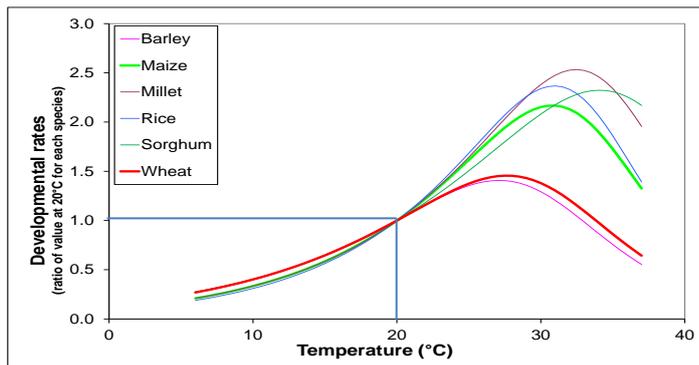


Fig. 1. Responses to temperature of leaf growth and of variables involved in leaf development (appearance rate, initiation rate, cell division rate) in maize and wheat (bold), and related cereals. Because this response involves different processes with different units, all rates are normalized to 1 at 20°C. From Parent et al. (2012).

An essential point is that temperature responses differ between species but not between genotypes of a given species. The domain of validity of the model and the conclusion of an absence of genetic variability are associated with limits, namely (i) the range of temperatures in which responses are reversible (typically 6-35°C for maize and wheat) (ii) temperature scenarios in which cold or hot temperatures occur during a few hours in the morning (cold) or afternoon (hot). The model therefore applies to the range of temperatures and durations of exposures to low or hot temperature that are compatible with the area of adaptation of the studied species.

1.1.2 Hydraulic processes control changes in leaf elongation rate with water deficit.

Plants are constantly facing rapid changes in evaporative demand and soil water content, which affect their water status and growth. Leaf elongation rate rapidly declines in the morning and recovers upon soil rehydration with half times of typically 20-30 min with a large genetic variability, thereby causing appreciable genotypic differences in final leaf area.

In continuous light, leaf growth rate oscillates following oscillations of plant hydraulic conductivity and of the transcripts amount of aquaporin genes. These oscillations, depending on the circadian clock, have an amplitude that depends on the level of stress during the previous days in naturally fluctuating conditions (Caldeira et al. 2014b). This shows that temporal changes

in water transfer in the plant are a major determinant of leaf growth rate, and allows calculation of circadian-based changes in plant hydraulic conductance.

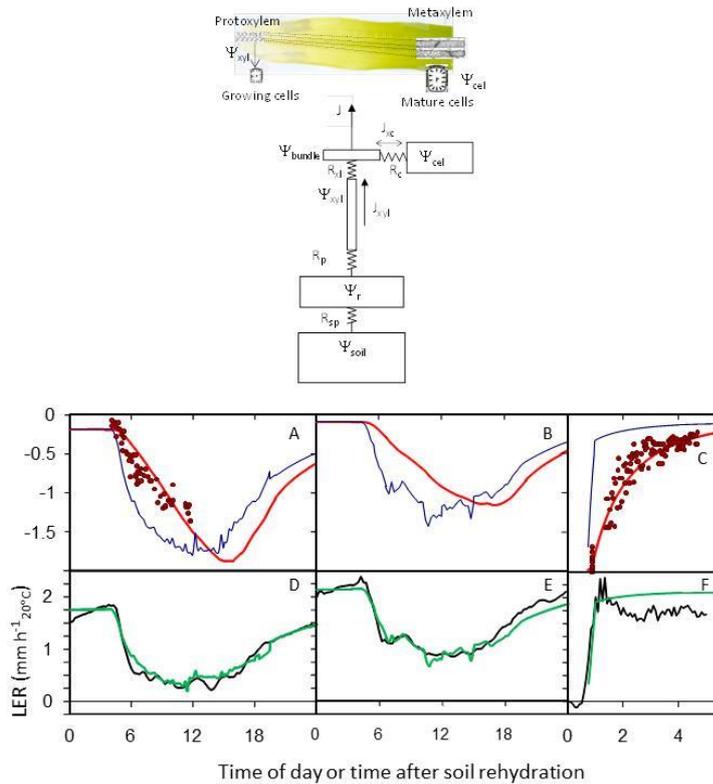


Fig. 2. Schematic representation of the model and its outputs if run with environmental conditions of two days with high (panels A D) or low (panels B E) evaporative demands or in a rehydration experiments (panels C F). Panels A to C: red line, leaf water potential (Ψ_{cell}); blue line, xylem water potential (Ψ_{xyl}); red circles, leaf water potential measured experimentally (Ψ_{leaf}). Panels D to F: green and black lines, simulated and observed leaf elongation rates (LER), respectively.

A hydraulics-based model with genetic inputs predicts leaf growth, transpiration and water transfer taking the stress hormone ABA into account (Tardieu et al. 2015). Briefly, water flows following gradients of water potential from soil to roots, xylem and bundle sheaths. The flux at bundle sheaths either contributes to transpiration or is diverted to mature leaf cells, depending on the direction of the gradient of water potential. Hydraulic conductances on the water transport depend on circadian and environmental effects. The water channel aquaporin PIPs are involved in (i) the changes in hydraulic conductivity associated with environmental conditions and with the circadian rhythm of the plant, (ii) the genetic variability of sensitivity to water deficit in the DROPS panel. The resulting model correctly predicts scenarios with sequences of fluctuating and stable conditions of light, thereby testing both origins of hydraulic conductivity. It adequately predicts leaf elongation rate in 10s of climatic scenarios (Caldeira et al., 2014a).

Hence physiological approaches in phenotyping and omic platforms allow us to propose that the genetic variability of the sensitivity of leaf growth to water deficit is linked to hydraulic processes.

1.2 Grain Abortion

Grain number is a major yield component in maize and wheat. It is mainly determined slightly before and around flowering time in both species, via a time series of events. The outcome of the interplay between physiological processes and the timing of stress dynamically determines the final set of grains contributing to yield.

1.2.1 Grain abortion of maize in water deficit depends on silk growth.

Silks are carried by each ovary and collect pollen for ovary fertilization. They grow with a pattern close to that of leaves, with maximum growth during the night and minimum growth during the day. Day-night oscillations increase at high evaporative demand and under water deficit, as they

do in leaves. We have shown that silk and leaf growth sensitivities are highly correlated in a panel of genotypes, and that half of QTLs are common between both sensitivities.

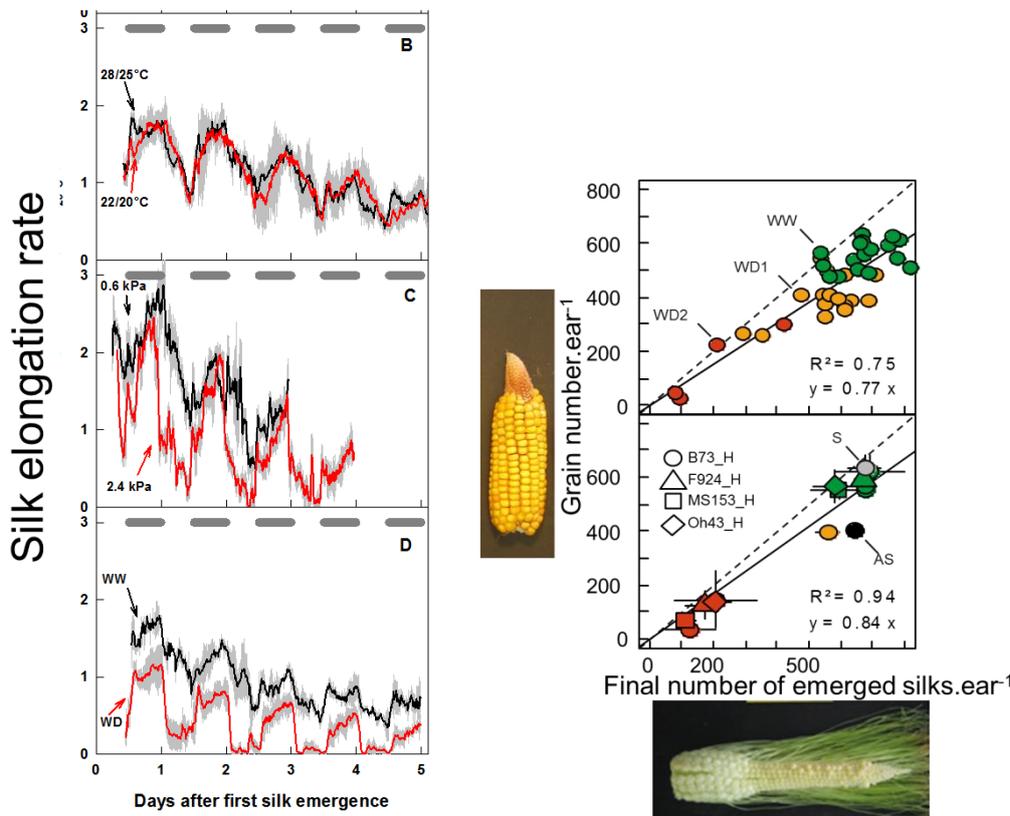


Fig. 3B-D. Time course of silk elongation rate with day-night oscillations. These oscillations increase with evaporative demand (C) and water deficit (D).

Fig. 3E, Grain number at harvest is closely related to the number of emerged silks at flowering time, this number is determined by silk elongation rate. Upper panel, individual plants of one genotype. Lower panel, different experiments with four hybrids. The correspondence between symbols and hybrid is indicated in the figure.

Silk elongation rate is closely related to ovary abortion via the timing of silk extrusion (Oury et al., 2016a). Silks are initiated sequentially along the ear, most basal silks being the oldest. Youngest silks emerge last, and are considerably delayed in case of water deficit. Abortion occurs in ovaries that carry a silk that emerges more than two days before the end of silk growth. This model applies to different genotypes, different experiments, explains the base-tip gradient of abortion, and accounts for abortion rate caused by water deficit. The mechanisms presented above have been simplified and included in the APSIM crop model that adequately accounts for abortion in the field (see §3).

1.2.2 There is no need for metabolic indices for predicting grain abortion

A thorough metabolic analysis has been carried out to test whether metabolic indices could increase the quality of prediction of grain abortion and yield, thereby allowing to predict grain yield from linear combinations of metabolite concentrations. To our own surprise, the carbon status of ovaries and of silks are very similar in plants subjected to well-watered or water deficit treatments. This study is based on a transcriptomic analysis of both organs, on the concentration/amount of metabolites and on activities of key enzymes (Oury et al., 2016b). In contrast, genes related to expansive growth and to water transport are differentially expressed in well-watered and stressed plants at the onset of water deficit. Hence, the metabolic analysis reinforces the conclusion that hydraulic processes and silk growth are probably essential for grain abortion.

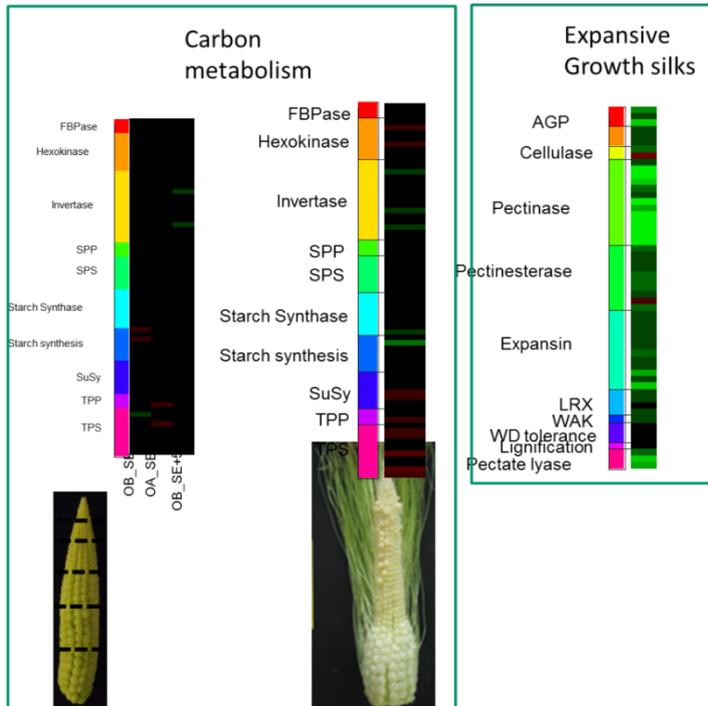


Fig. 4. Transcriptomic analysis of young ovaries and silks. Each figure shows the differential expression of genes, either over expressed in well-watered plants (green) or in water stressed plants (red). The left panel shows the differential expression of genes involved in carbon metabolism in both ovaries and silks (no significant differences in expressions). The right panel shows differential expression for genes involved in mechanical cell wall properties, which control expansive growth.

1.3. Root System Architecture

Deep roots can improve plant response to drought. However, simulations in a wide range of soil depth and climate scenarios indicate that increased rooting depth does not always lead to a yield advantage under drought conditions (see §4) . In DROPS, we adopted an architectural view that embraces root architecture, root hydraulics and soil hydraulics (Draye et al., 2010).

1.3.1 A nearly constant root elongation rate for all roots of a plant.

DROPS experiments in aeroponics have revealed that individual roots (primary, seminal and adventitious) keep a constant growth rate, determined at the time of their emergence. This suggests that roots which emerge later are the one that access assimilates in priority, and that the sources and sinks are geared in a way that allows roots to grow at their full potential.

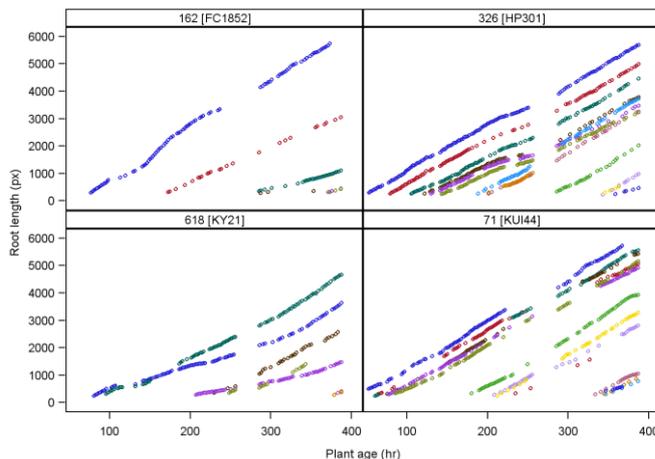


Fig. 5. Elongation of the first order roots of four maize plants grown in the aeroponics platform. The figure reveals the stability of root elongation rate that suggests that individual roots grow at their full potential when unimpeded, and that this potential is defined at the root level at the time of their emergence.

From the same experiments, we have found that the rate of emergence of successive adventitious roots was nearly constant. This additional result suggests that root emergence overrides root elongation in terms of allocation of assimilates. These two results considerably simplify the analysis of root system growth.

1.3.2 A dynamic structural model to predict maize root system architecture.

We have exploited the above results to simulate the backbone structure (primary, seminal and adventitious) of the root systems of the whole panel. We have developed a data analysis pipeline that estimates the mean and standard deviation of the main parameters of the root model (RootTyp) that differentiate the 250 hybrids.



Fig. 6. Root systems simulated with RootTyp using extreme parameter values in the maize panel. Left: effect of root elongation rate. Right: effect of insertion angle on the stem base.

1.3.3 A simplified model to predict patterns of water uptake and soil water availability based on the hydraulic architecture of the soil-root system.

Water uptake follows simple rules of passive flow driven by water potential gradients and following paths of lowest resistance. The model R-SWMS explicitly simulates the water flow in the soil-root continuum down to the organ level. Considering that the root axial resistances are much lower than the root radial resistances, the flux equations in the root system can be drastically simplified (Couvreur et al., 2012). Under these conditions, the radial volumetric flow rates are given by the sum of two processes: (i) the water uptake under uniform soil water potential conditions and (ii) actual transpiration under heterogeneous soil conditions according to a global root system conductance.

1.4. Transpiration Efficiency (TE)

Biomass accumulation is intrinsically linked to transpiration because stomatal aperture and leaf area determine the rate of both processes. There is therefore an inherent conflict between biomass accumulation and stress avoidance *via* reduction of transpiration. However, the ratio of biomass accumulation to transpiration has an appreciable genetic variation that has been evidenced in the phenotyping platforms in Lancaster and in Montpellier. We did not succeed in establishing a stable genetic relationship between transpiration efficiency and hormone production in panels of maize or wheat genotypes, as hypothesized in the project. This has been tested by measuring the stress hormones abscisic acid (ABA) and ethylene, the latter being characterised via its precursor ACC. Both hormones have been measured in panels of 100 genotypes of wheat and maize, repeated with 250 maize hybrids. Weak relationships have been observed between TE and individual concentrations of ABA and ACC, or with the balance between ABA and ACC. These relationships were not repeatable between experiments. Hence, we conclude that genetic variations of TE do not follow changes in hormonal balances in a straightforward way. The work continues to identify if more subtle relationships may exist. The APSIM crop model has considered the direct measurements of TE in each genotype (Lancaster and PhenoArch) as parameters of the model.

1.5. Conclusion

The physiological and genetic analyses performed in phenotyping and omic platforms have allowed substantial progress for our understanding of the genetic variability of the four target traits. As expected, several hypotheses stated in the project could not be accepted, in particular those involving relationships between yield or TE and linear combinations of concentrations of hormones or metabolites. In contrast, the mainly hydraulic origin of the genetic variations of the

sensitivity of growth to water deficit, the complete change of view for the genetic and physiological control of grain abortion (from carbon driven to hydraulically driven mechanisms), and the considerable simplification for root growth rate in a root system are major findings that profoundly change our vision of genetic controls of plant responses to environmental conditions. They have been formalized into detailed models that have then been included, after simplification, in the crop model algorithms in such a way that allelic variants affecting each trait can be translated into changes in yield across European climatic scenarios.

2. Contribution of genomic regions and candidate genes to traits and yield

2.1 Genetic analysis and QTLs in the maize panel

2.1.1 A dent panel of hybrids, densely genotyped (1 M markers)

The maize DROPS panel belonged to the dent group, with lines selected for a range of flowering time within 10 days, and crossed with a common flint tester. The panel was first genotyped with a chip of 50,000 SNP markers as stated in the project. Technological evolutions made it possible to extend genotyping with a larger marker array and Genotyping by Sequencing (GBS). These two techniques resulted in 354,000 and 722,000 polymorphic markers, respectively. An analysis of linkage disequilibrium (LD, statistical association between alleles of different loci) showed a clear decrease with the genetic distance between loci. A LD with $R^2=0.1$ was reached at a distance of 0.03 cM. This corresponds to a physical distance of 90 kB, with a strong variation between centromeric and telomeric regions. These values of LD were considered when searching candidate genes putatively causal of the associations presented below.

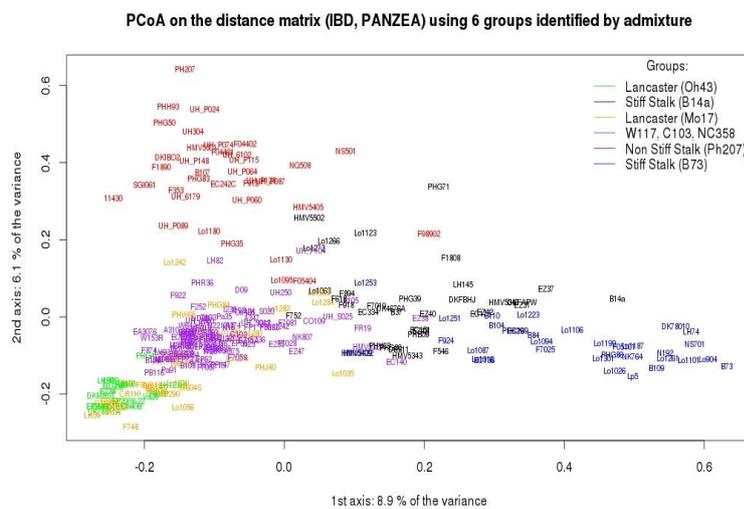


Fig. 7. Main lines of population structure within the DROPS maize panel, as revealed by Principle Coordinate Analysis of 50k SNP data. Colors refer to groups identified with software ADMIXTURE. Main trends appear related to the main ancestors of the different breeding pools (e.g. Ph207 and B73).

The panel was clustered in six groups representing the main dent maize origins, with sufficient overlap between groups and diversity within groups to allow sufficient power in GWAS. Considering marker-based relatedness was mandatory to control false positives. The power of association tests on a given chromosome was increased by 40% by estimating relatedness in the other nine chromosomes only (Rincent et al., 2014). In terms of software, FASTLMM proved to be the most efficient both computationally and in terms of statistical properties (exact model). A R pipeline based on this principles was implemented to conduct single trait and single environment analyses within the project.

2.1.2 QTLs in the field displayed contrasting allelic effects depending on climatic scenarios

The panel was phenotyped in 29 experiments (combination site x year x water regime) covering a west-east transect in Europe, plus one location in Chile (col. PIA project *Amaizing*). Detailed environmental conditions were recorded in each situation, including meteorological data and soil water potential recorded at three soil depths. Three scenarios of soil water status, identified via simulations over Europe (see §4), were considered for classifying experiments. Three scenarios of temperature were identified, depending on night and maximum temperatures.

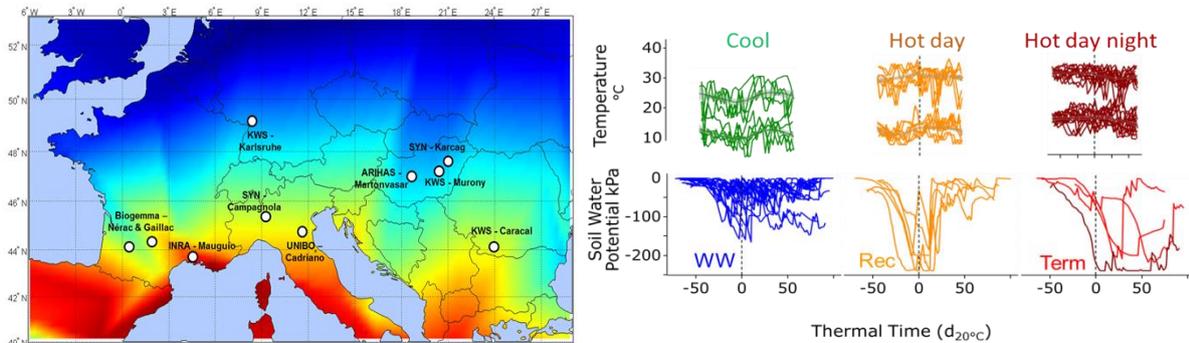


Fig. 8. Network of field experiments for maize in Europe. Each site involved experiments for two years with two treatments (rain fed and irrigated). Right panels, environmental scenarios based on time courses of temperature (upper panel) or soil water potential (lower panel) during 20 days around flowering time. Each line corresponds to one combination site x year x water regime in which an experiment was performed.

Grain yield ranged from 1.2 to 12.9 t ha⁻¹ for the reference hybrid (1.5 to 11.2 t ha⁻¹ for the mean of the panel) in the 29 experiments. As expected, highest yields and grain numbers were observed in WW situations with cool temperatures (10.8 t ha⁻¹ average for the reference hybrid) while situations with soil water deficit and high temperature had lowest yields (4.7 t ha⁻¹ on average). Within WW situations, grain yield tended to be much lower in hot than in cool situations, with a lesser effect on yield in situations with hot maximum temperatures but cool temperatures during the night. The panel showed a large genetic variability of yield and yield components. Within each situation, the coefficient of variation of grain yield, representing the genetic variability, varied between 0.11 and 0.46 (0.12 and 0.52 for grain number and 0.04 and 0.12 for grain size). Heritabilities ranged from 0.39 to 0.91 in the different situations with a median of 0.56.

The combined multi environment and single-environment GWAS led to the identification of 62 QTLs for grain yield after grouping significant SNPs that were in strong linkage disequilibrium. QTLs of grain number are very similar to those of yield. As in most analyses of multi-site networks, QTLs were unstable. One QTL involved 10 experiments, two involved 6 experiments, and 7 involved two experiments. All identified QTLs showed a significant interaction with environment, showing that all allelic effects depended on environmental conditions.

For example, one QTL was observed only in those situations with hot air and high evaporative demand during the period encompassing flowering. Another QTL was observed in wet soil (soil water potential from 0 to -0.1 MPa, but disappeared when the soil was drier (soil water potential lower than -0.2 MPa). Overall, ten QTLs can be interpreted as QTLs of tolerance. Six of them showed largest effects in scenarios with high temperature, and small or no effects in cool temperature so they can be considered as bringing tolerance to high temperatures. Four QTLs were observed in dry soils but not in wet soils, so they can be considered as QTLs of tolerance to drought. Other QTLs were observed in cool temperatures or wet soil but disappeared at high temperature or dry soil so they can be interpreted as QTLs of high sensitivity to stress.

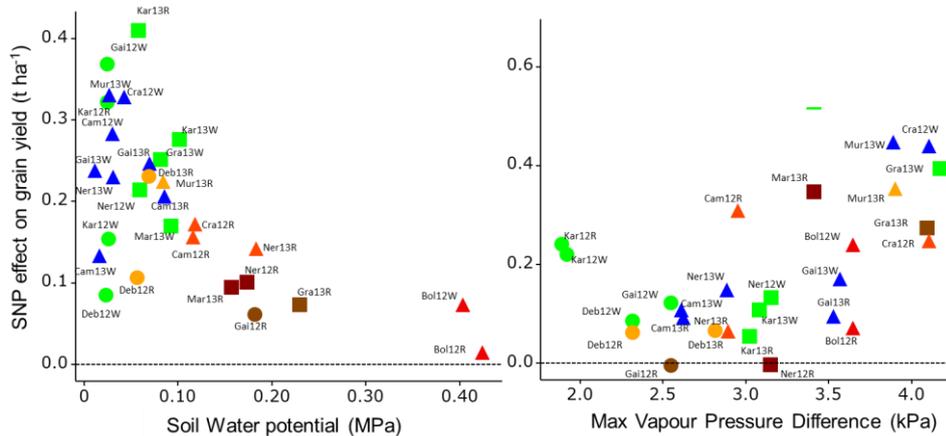


Fig. 8. Allelic effects at two QTLs on chromosomes 3 and 6, as a function of environmental conditions. The Y axis represents the difference in yield between lines carrying the allele of the reference line B73 and those carrying the other allele. In the left panel, the x axis is the mean soil water potential measured at flowering time (see Fig. 1). In the right panel, the x axis represents evaporative demand (vapour pressure difference) during the same period. Each point, coupled value of SNP effect on yield and soil water potential (left) or vapour pressure deficit (right). A common colour between two sites indicates that situations were classified in a common environmental scenario.

2.1.3 QTLs in phenotyping platforms were associated with important traits such as the progression of plant cycle, plant growth rate or sensitivity of growth to water deficit

Traits have been measured in the phenotyping platforms of Louvain la Neuve (roots), Lancaster (water use efficiency) and Montpellier (growth, plant architecture, sensitivity to water deficit).

- Traits associated with growth (biomass, leaf area) have been studied in 3 experiments of the PhenoArch platform (Montpellier), resulting in QTLs of plant ability to produce biomass. Phenotypic values have been used as parameters specific of each hybrid in the crop model (§3).
- Most interesting traits identified in the platform are those relating to the sensitivity of plant growth to water deficit, calculated via regressions of plant leaf area with soil water potential across experiments. Interesting QTLs have been identified, which colocalize either with aquaporin PIP transcripts, or with QTLs of sensitivity of yield identified in the field.
- Traits representing the progression of the crop cycle (phyllochron) resulted in QTLs allowing a better understanding of the genetic variability of plant development. They are a key element for the genetic architecture of parameters of the crop model.
- Traits involved in root growth and architecture have been evaluated in the phenotyping platform of Louvain La Neuve. Preliminary QTLs, calculated with the 50K genotyping have been identified. We expect much better results with denser genotyping.

2.1.4 Combined QTLs in phenotyping and omic platforms form an unprecedented dataset for understanding the roles of candidate genes behind QTLs in the field or in phenotyping platforms

Samples of mature leaves and of growing regions of leaves have been collected in the PhenoArch platform and in the field, for analyses of 10 metabolites, of the growth hormones abscisic acid (ABA), of a precursor of ethylene, ACC, and of ^{18}O , an indicator of whole plant transpiration. The amounts of transcripts of five aquaporin PIPs have also been measured in all genotypes of the panel. Finally a proteomic study has been performed on the same plants in the context of a companion project (ANR-PIA Amaizing). The most interesting dataset is at this stage that of PIP aquaporins, whose amounts are associated with very strong QTLs located either on the positions of the corresponding gene (cis QTLs) or in other positions in the genome (trans QTLs). The latter

will provide novel candidate genes that can modulate aquaporin transcription which has shown to be closely linked with leaf growth and abortion. Several QTLs of aquaporin transcripts co-localize with QTLs of sensitivity to water deficit in the phenotyping platform, but also in the field. These colocations are essential to understand the genetic variability of the sensitivity of growth and yield to water deficit.

2.1.5 Conclusion for the maize study

The genetic analysis of field, platform and omic QTLs results in a very large dataset that has potential for major discoveries. In particular, we can now model the allelic effects at QTLs as a function of specific and easily measurable environmental conditions. This is essential for identifying which alleles will be favourable in which European regions. Several colocations between transcript amounts, measured sensitivity in platforms and yield in the field present extremely promising avenues for linking the genetic variability of yield with candidate traits, QTLs and genes. Corresponding QTLs will be tested with near isogenic lines either already available or that have been developed in the context of DROPS. This will be performed in the companion ANR-PIA project Amaizing.

2.2 Genetic analysis and QTLs in the durum wheat panel.

2.2.1 Organisation of molecular data for genome wide association studies (GWAS)

The durum wheat panel included 188 genotypes (mostly cultivars and advanced lines) representative of the breeding germplasm from Mediterranean Countries, South-Western USA, breeding programs at CIMMYT and ICARDA. It involved mostly Mediterranean-adapted germplasm released between 1970s and early 2000s, semi-dwarf with wide range of yield potential and yield component variation. The panel was initially genotyped with 300 SSR markers and, in the DROPS project, densely genotyped with 900 DArT and a 90,000 SNP array (transcript associated SNPs), 19,815 of which were polymorphic. This led to a high genetic coverage and resolution. A high-density tetraploid consensus map integrating SSR, DART and SNP has been obtained from 13 linkage maps of *T. durum*, *T. dicoccum* and *T. dicoccoides*. In total, 19,815 markers showed polymorphism reduced to 13,823 when filtered. As based on the tetraploid consensus map, only few hundreds informative SNPs remained unmapped and were not considered for further analysis. SSR markers, due to their allelic richness, were mostly used to estimate the panel population structure and kinship matrix. Population structure was estimated based on 96 independent, informative and well distributed SSRs while kinship matrix was calculated based on the identity-by-state genetic similarity matrix among accessions from the whole set of 300 SSRs. Linkage disequilibrium analysis based on the high-density SNP map confirmed a decay to the threshold value of $r^2=0.3$ at 2.20 cM on average. This distance has been used to set a confidence interval for the GWAS analysis. The LD distribution along chromosomes showed relatively uniform genome coverage, and the presence of LD-blocks where either the LD decay rate is slower than in other regions or the SNPs are present at higher density.

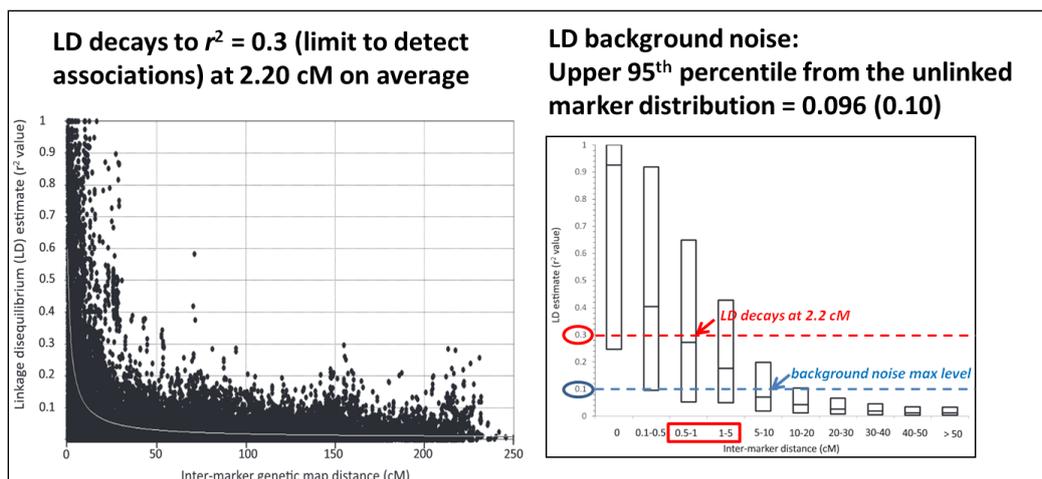


Fig. 9. Linkage disequilibrium decay rate in the Durum Panel. Left: scatterplot of LD values (r^2 values) as a function of genetic map distances. Right: box-plot of LD r^2 values by classes of map distances. By fitting the equation relating LD, recombination rate and population size the LD decay to the threshold of $r^2 = 0.3$ was estimated at 2.20 cM distance while the background LD due to population structure was estimated at a maximum of $r^2 = 0.1$.

2.2.2 Field QTLs in the durum panel

The Panel was phenotyped in 10 experiments in Italy, Hungary and Turkey, for two consecutive years (2012 and 2013) and two water regimes (rainfed and supplemental irrigation). Detailed environmental conditions, including soil water content at key developmental stages, have been recorded. Average grain yield ranged from high (10.4 t ha^{-1}) to medium-to-low ($1.2\text{-}3.6 \text{ t ha}^{-1}$). Grain size and number of fertile tillers per square meter showed patterns that followed yield. Heritability values were high for phenology traits (h^2 values between 0.73 to 0.93, median = 0.86), medium for yield (0.22-0.71, median = 0.49), medium-to-high for the yield component grain size (0.49-0.95, median = 0.78) and medium-to-low for fertile tillers (h^2 range = 0.33-0.47). A large component of environmental interaction has been observed for most traits.

Major genes affecting phenology and plant height have been considered as controls in the GWAS analysis of yield and yield components. Re-sequencing has been used to identify novel variants (associated to flowering) known to be present in the Durum Panel. The allelic variants of major genes have been tested for association with the traits recorded in the field trials and highly significant associations have been found, particularly for *PPD-A1*, *PPD-B1* and *RhtB1b*. Based on these results, association have been carried out by considering the major molecular variants at all these flowering time genes as covariates in the GWAS tests for yield.

Robust QTLs were identified through single environment GWAS for heading date, grain yield and grain size. The number of uniquely identified QTLs was similar for these three traits (94, 107 and 98, respectively). Highly heritable traits such as heading date and grain size showed QTLs with more significant associations across environments than grain yield. The B genome was consistently enriched in QTLs as compared to the A genome. For example, 187 QTL-environment associations were observed for the B genome vs 100 associations for the A genome. For yield, as an example of low-heritability trait with a high component of environmental specificity, 66 QTL showed significant associations but 7 QTLs only were significant experiment-wise. There were numerous cases of co-location between QTLs for early vigour, flag leaf chlorophyll content and grain yield and yield components as well as between heading date and grain yield QTLs.

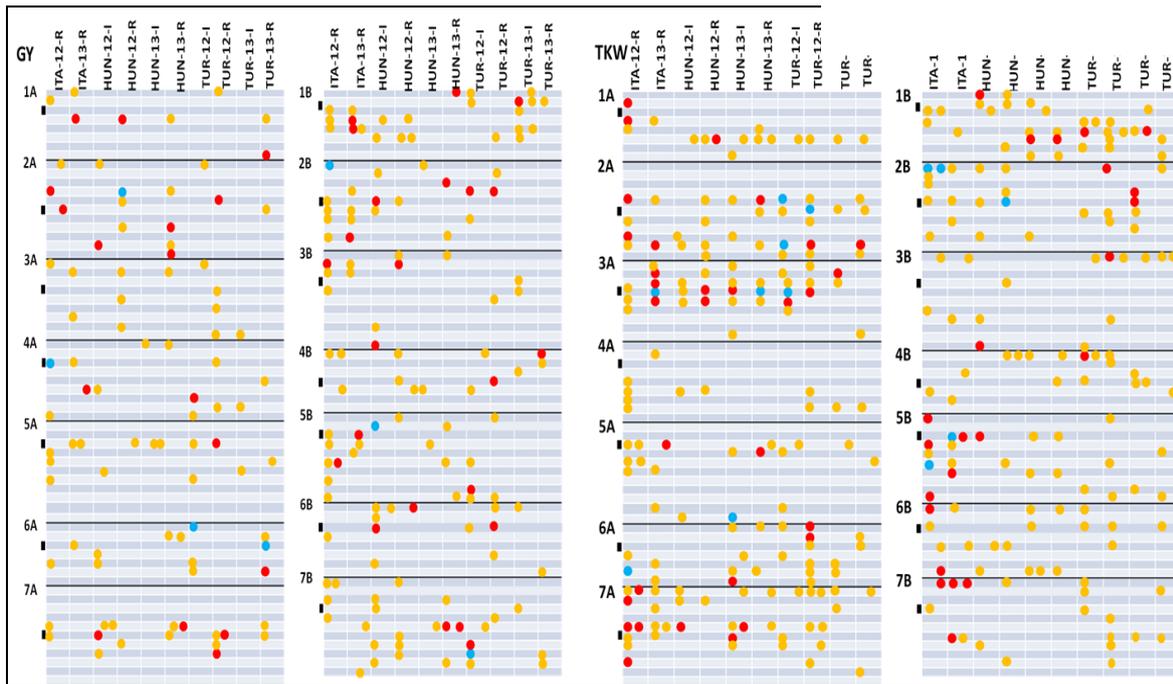


Fig. 10. Positions and magnitude of QTLs for grain yield (GY) and grain size (TKW) for each of the 10 studied situations. QTLs are reported as circles in a bin-grid of 20cM/bin. Orange circles: QTL significance at $P < 0.01$, marker-wise; Red circles: QTL significance at $P < 0.001$, marker-wise; Blue circles: Bonferroni-corrected QTL significance, $P < 0.05$ exp-wise.

2.2.3 QTLs for root system architecture

The Durum Panel, together with two durum recombinant inbred line populations, have been characterized for root system architecture traits at the seedling stage. The same SNP/DaT genotypic data set has been used for a GWAS analysis that evidenced 20 QTLs for root length and number as well as 30 QTLs for root angle. Based on their relative additive effects, allelic distribution and co-location with QTLs for kernel weight and yield, the root QTLs have been prioritized in terms of breeding value. Three major QTL clusters for root length and number and five QTL clusters for root angle appear particularly valuable for further characterization towards their positional cloning and possible deployment in marker-assisted selection.

2.2.4 Conclusion for the durum wheat study

This analysis has shown that the level of informativeness and precision of GWAS in tetraploid wheat has greatly increased compared to the standard previously reached with the SSR and DaT marker methodologies. Moreover, the identified QTLs are defined within smaller intervals as compared to analysis in recombinant inbred lines and are genetically anchored to well-defined regions (based on the transcript-associated SNPs) of the wheat genome. As in maize, most of QTLs identified for grain yield showed a high level of environmental specificity but QTLs for other more heritable traits such as canopy photosynthetic capacity and grain size are more amenable to fine mapping and breeding exploitations as single QTLs or for wide genomic applications such as genomic selection.

3.3 Genetic analysis and QTLs in bread wheat.

3.3.1 Common QTLs were identified for plant growth and transpiration rate in the phenotyping platform and for yield in the field

The Gladius/Drysdale population developed in ACPFG from Australian lines contrasting in their mechanisms of yield maintenance under water deficit was studied in both a phenotyping

platform (The Plant Accelerator, Australia) and in the field under drought and well-watered conditions. Biomass and leaf area were estimated via image analysis. A total of 20 QTL were identified for growth traits, transpiration and water use efficiency in a drought experiment in The Plant Accelerator. From the 20 QTLs found for several traits in the platform, some showed strong effects, accounting for between 26 and 43% of the variation on chromosomes 1A and 1B, indicating that the G×E interaction could be reduced in a controlled environment and by using dynamic variables. A total of 44 QTL were identified in the population in well-watered and drought treatments in the semi-controlled conditions in a field equipped with irrigation and a rainout shelter. Co-location of QTLs identified in the platform and in the field showed a possible common genetic basis at some loci. Interestingly four regions show overlap between QTL for growth rate and yield component in the field.

Table 1. Overlapping QTL between traits identified in the imaging platform and the semi-controlled field experiment (polytunnel). Blue indicates QTL found under well-watered conditions, red under drought.

	Chromosome arm	1BL	2AS	2AL	4BL	5BL
Imaging platform QTL	LER _{AVE}	Blue				Red
	Growth _{AVE}	Blue	Blue	Blue		Red
	LER.Tx					Red
	TR	Blue			Red	
	WUE			Red	Red	
Polytunnel QTL	Yield		Blue	Blue		
	Grain number			Red		
	Grain weight			Blue		
	Harvest index					Blue
	Spike number	Blue				
	Tiller number					Red

3.3.2 QTLs for metabolites in field under low rain-fall environment

A total of 700 metabolites were analysed by GC-MS and LC-MS in samples of flag leaves of the Excalibur/Kukri population grown in South Australia field conditions. 558 QTLs of metabolite were identified. Comparison of the agronomic and metabolic trait variation uncovered novel correlations between some agronomic traits and the levels of certain primary metabolites, including metabolites with either positive or negative associations with plant maturity-related or grain yield-related traits. Our analyses demonstrate that specific regions of the wheat genome that affect agronomic traits also have distinct effects on specific combinations of metabolites.

3.3.3 Fine mapping of a QTL for yield revealed contrasting effects in different drought scenarios

The Australian population RAC875/Kukri was studied in 23 field trials and under semi-controlled conditions using irrigation and rain-out shelter for dissecting the effects of QTL on chromosomes 3BL and 7AL across conditions. The RAC875 allele increased all yield components and plant biomass in environments characterized by deep soil and under dry and hot conditions such as New South Wales and Mexico. It had a significant negative effect in mild drought scenario using irrigation. It was not significant in fields with shallow soils of southern Australia. Instability of QTLs of yield under water deficit, observed in maize and durum wheat, was therefore clearly demonstrated for bread wheat.

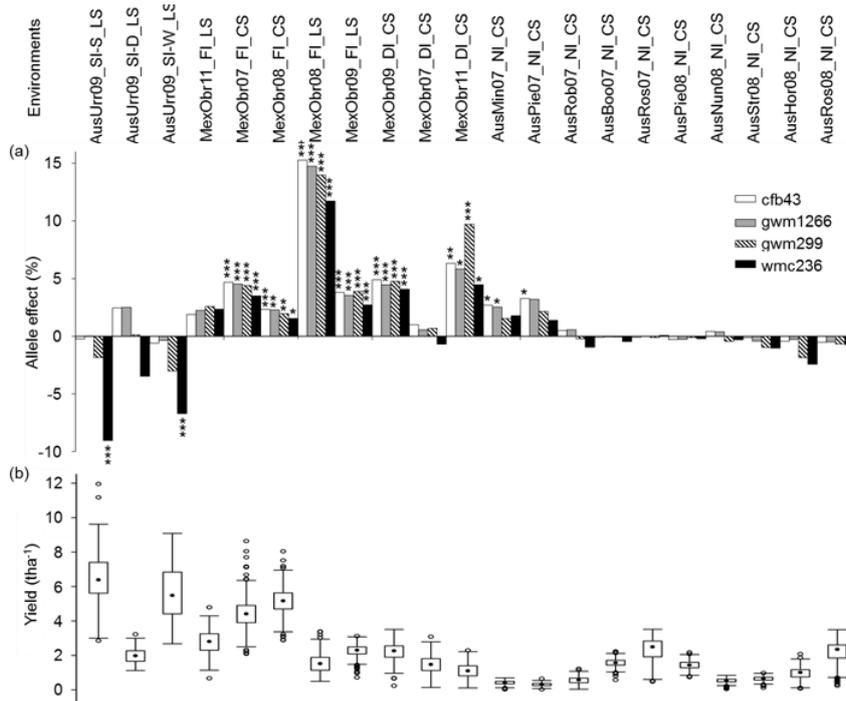


Fig. 11. Effect of 3B QTL on yield under different drought scenarios in Australia (Aus) and Mexico (Mex). Urr: mild drought scenario using irrigation in shallow soil of Southern Australia. Obr: deep soil of Obregon under hot and dry climate. Min, Rob, Boo, Pie, Nun, Str, Hor, Ros: severe drought scenario in shallow soil of Southern Australia.

3.3.4 Introgression of QTL for improving yield in dry environments into elite Turkish lines.

The QTL presented above was successfully introgressed into five elite wheat lines from Turkey using markers from the fine mapping (U Sabanci). Field tests enabled to select five BC3F2 lines with the region of interest. Yield data have been collected for 2015 field trials and are being analysed. This material will be an important resource for demonstrating the value of the studied QTL for breeding in different Mediterranean environment.

3.3.5 QTL for improving yield in Hungarian germplasm grown in different water regimes

The Plainsman/Magma population has been studied in field over 4 years under two water regimes in Hungary. Weather and soil data were recorded for each trial. A total of 65 QTL were identified. Most QTL were consistent across the environments and explained only a small portion of the phenotypic variance individually. The largest effect QTL were those showing significant interactions with the environment. QTL of osmotic stress response found in controlled conditions overlapped with the field QTL in 6 chromosome regions.

3.3.6 Conclusion for the bread wheat study

The whole journey from QTL discovery to breeding has been performed in the bread wheat action of DROPS. It included QTL detection, QTL analysis with omic methods, analysis of the QTL effect on yield over a large range of environmental situations and introgression of the involved genomic region in Turkish elite lines for breeding purpose. Overall, the conclusions are similar to those in the other two species studied in DROPS, namely that QTL effects largely depended on environmental conditions. In this case, a given QTL had an effect on yield ranging from -8 to +15%. The conditions in which positive and negative effects occur have been identified, so positive effects of wheat QTLs can be associated with different regions in Europe (see §4).

3. A new-generation crop model for predicting allelic effects

Crop models can aid breeding programs to select appropriate genotypes in each geographical region, by performing hundreds of simulations in different sites for tens of years, i.e. a number of scenarios that will never be compatible with multi-site networks of field experiments as those presented in §2. However, many crop models lack the functionality to do this as they do not

adequately predict plant phenotype as an emergent consequence of genotype x management x environment interactions for key adaptive traits. While these models are structured adequately for agronomic applications, such as examining effects of sowing date or planting density, their use in breeding require prediction of the complex adaptation landscape arising from interacting traits in a range of scenarios (Messina et al., 2011). Enhancing crop modelling capability for use in genetics requires algorithms that explain complex phenotypic responses, rather than rely on algorithms that statistically describe them. We have adopted this approach in DROPS by advancing the modelling framework in the software platform APSIM. APSIM is a modular process-based cropping systems simulation model, developed in Australia. It simulates the yield of field crops in response to variation in environmental (climate, soil) and management conditions, either as individual crops or as part of a rotation. One of the modules in APSIM is a generic crop model that contains a library of routines for crop growth and development processes. The APSIM maize module has been redesigned for a use in genetics. A similar work has been performed for the SIRIUS-quality model for wheat in a companion project by P. Martre (INRA Montpellier), thereby allowing simulations with wheat in §4.

3.1 Growth Maintenance

3.1.1 A novel module integrates hydraulic knowledge presented in §1. The current module of leaf growth in APSIM has been replaced by a specific module that captures the essential characteristics of models presented in §1 and is consistent with the current level of complexity of APSIM. The response to temperature is similar to that in Fig. 1. The effects of evaporative demand and soil water deficit on leaf elongation rate (LER) are represented by a simple equation

$$\text{LER} = a + b \text{VPD} + c \Psi_{\text{soil}} \quad (\text{eq. 1})$$

where VPD is air pressure vapor deficit and Ψ_{soil} is the mean soil water potential. a , b and c are parameters that characterise each genotype and can be represented by a sum of QTLs, thereby allowing us to use the model in a genetic context. Eq. 1 is similar to the equations which drives leaf elongation rate in the model of Fig. 2, except that the latter considers the effect of xylem leaf water potential instead of the individual effects of VPD and Ψ_{soil} . The formalism of Eq. 1 has been kept to avoid the difficulty of using hydraulic equations, not feasible in the current version of APSIM. This algorithm for leaf elongation rate is interfaced with a model that predicts the timing of the development of each individual leaf of any genotype, regardless of its precocity for application to the DROPS panel. This allows prediction of the increase of leaf area along time, depending on environmental conditions and allelic values at QTLs.

The main parameters of the model (leaf appearance and ligule appearance) have been determined for all lines of the DROPS maize panel in the PhenoArch platform (Montpellier). We have checked that the programme of development established for the reference hybrid of the panel is common to the platform and to 8 fields across Europe.

The resulting model has allowed us to simulate the effect of QTLs of sensitivity of leaf growth to soil water deficit and evaporative demand in a large range of climatic scenarios through their impact on the leaf area index of the canopy. Because QTLs of silk growth are largely common to those of leaf growth, this model has the ability to be interfaced with the model of seed abortion presented below.

3.1.2 The resulting model has been tested in the DROPS network of field experiments. The model presented above has been tested on the reference hybrid of the panel for which DROPS partners have measured the final length and width of leaves in each field. The model was run in 15 fields by taking into account environmental data collected in these fields. Maximum leaf lengths (about 1 m) were observed in cool sites with low evaporative demand while minimum

lengths (about 80 cm) were observed in hot sites with high evaporative demand. The model simulations account for these differences across sites and years. This has allowed us to precisely simulate the effect on leaf growth of evaporative demand or water deficits measured in the field, for the 250 genotypes of the panel.

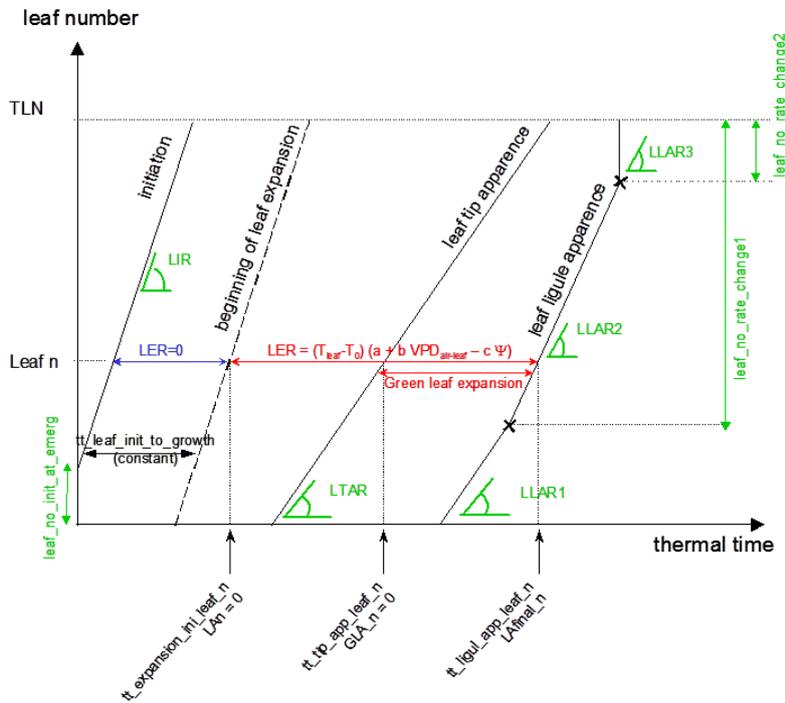


Fig. 12. Timing of leaf growth in whole maize and wheat plants. The model predicts the dates of initiation, beginning of leaf expansion, leaf appearance and end of growth (ligule appearance) depending on thermal time. The same model serves for wheat, but with one submodel per tiller.

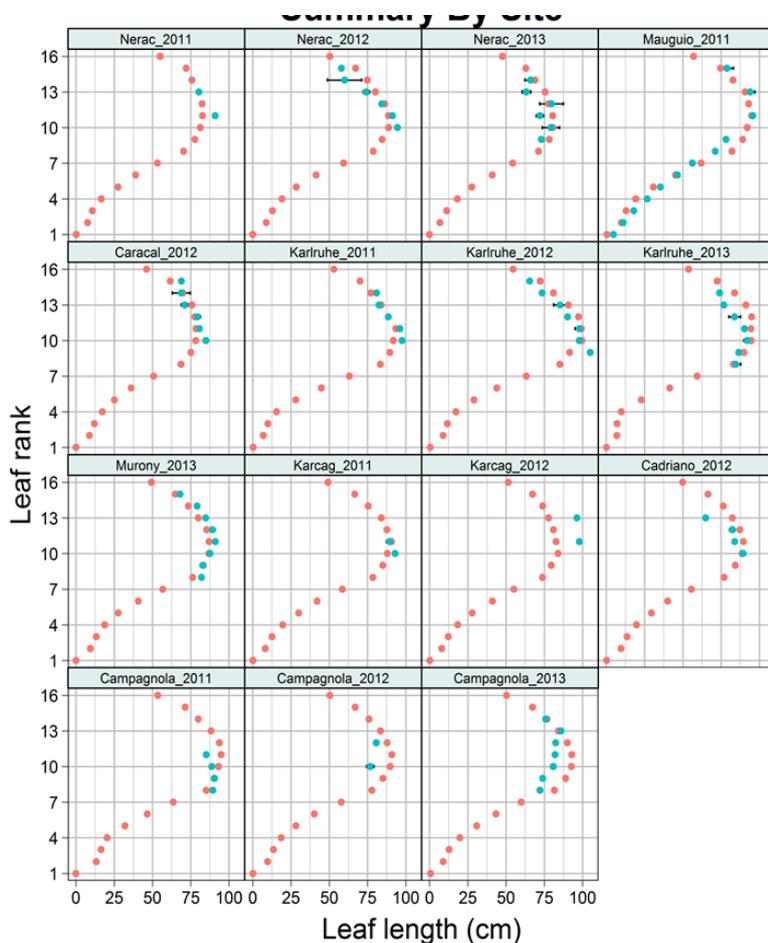


Fig. 13. Simulated and observed leaf lengths of all leaves of a plant (from oldest leaves at the base of the plant, 1, to youngest near the plant tip, 16) in 15 field experiments with contrasting conditions of evaporative demand. Red, simulated lengths, green, measured lengths.

3.2 Grain abortion

3.2.1 A novel module integrates the effect of silk growth presented in §1. We have implemented a module that simulates the development, growth and possible abortion of similarly aged cohorts of ovaries that turn into grains along the ear. This provides a dynamic framework for the development of every generation of grains on the ear, and for the dynamics of silk extension and the ear size. In this approach, elongation of silks and length of the husk determines the time it takes for silks to emerge from the tip and become receptive for pollination. Under well-watered conditions, silks appear early with a narrow age distribution, whereas under drought, appearance is delayed with wider age distribution and, eventually, a lower number of emerged silks, consistent with experimental data (§1.2).

Once silks have emerged, silk receptivity (aging), pollen viability, and pollen availability can reduce the number of silks fertilised, resulting in high abortion rate consistent with experimental data (§1.2). The potential grain number is determined from the number of fertilised silks, with non-fertilised ovaries aborting. Potential grain growth is determined from an expolinear function of potential grain size on thermal time, so further abortion can occur if this potential is not met. The derivative of the expolinear function provides the basis to model grain sink strength, that is, the ability of the grain to utilize reserves stored in vegetative organs prior to flowering time. This function determines a progressive ability of the grain to utilize reserves; only during the linear phase the grains become competent to fully utilize pre-anthesis stored carbon.

3.2.2 The model has been successfully tested. The model has been tested in a field without any rain (Chile) in which water deficits were applied at different times, either centred on flowering time or increasingly later in the crop cycle. Grain number was mostly affected by water deficits centred on flowering time while later deficits had a lesser effect. The model captured these variations and adequately predicted grain number and abortion. Grain size was increasingly affected by late water deficits in both the model and experimental data. The combination of the simulations of grain number and individual seed weight resulted in an adequate prediction of yield in the whole set of situations. Hence, the abortion module could be considered as adequate for performing large scale simulations over Europe. The model captures emergent behaviours, in particular the relationship between plant growth rate and grain number that underpins current models in different species.

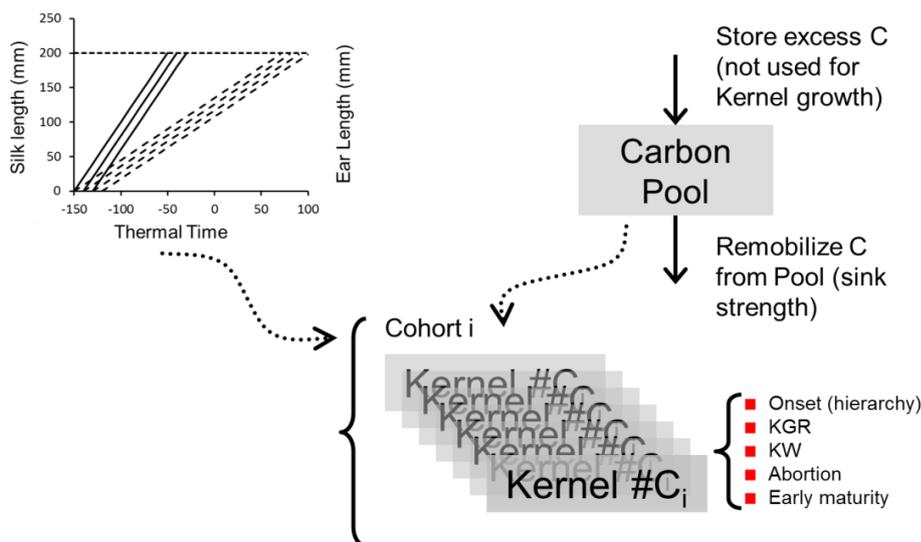


Fig. 14. Conceptual framework of the successive development of cohorts of ovaries and silks. Note the similarity with the development of leaves.

3.3 Root system architecture

We have adopted a simple functional-structural modelling approach focused on enhancing root architecture modelling in APSIM, based on integrating the 3D capabilities of RootTyp (See §1.3). In APSIM, roots grow to occupy soil space in an elliptical pattern dependent on key root system architecture specifications, such as angle of major seminal or nodal root axes. There is a constant root front velocity through the soil profile (if not impeded by inhospitable soil layers – dryness, salinity). However, it assumes that all soil space “behind” the expanding root front is effectively occupied by roots. While this is an obvious simplification of reality, it nonetheless enables prediction of water extraction by roots in a range of situations.

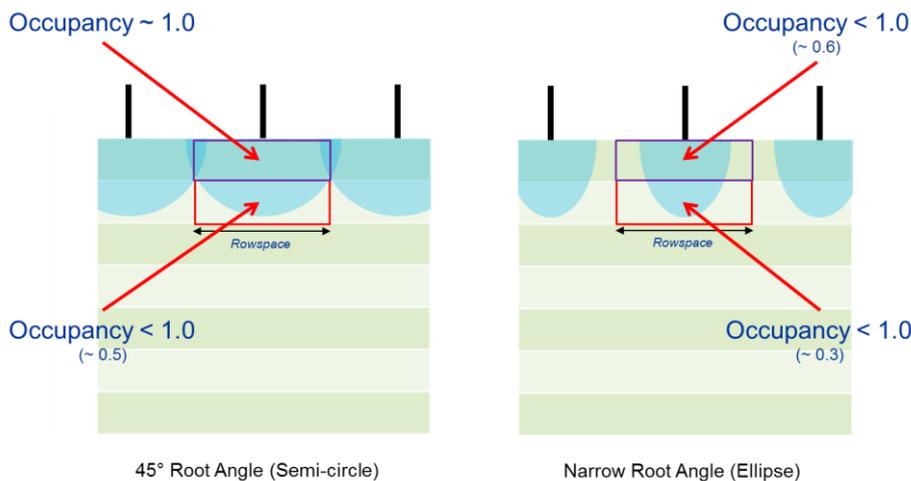


Fig. 15. Root system expansive growth and soil profile “occupancy” (Blue shading inside rectangles) as modelled for different main axis root angles in APSIM

We have created a prototype software platform that links RootTyp to APSIM by defining an effective distance from the root for water capture and then calculating a volume around each root to be considered as “occupied”. In this way the 2D occupancy factor of APSIM is converted to a 3D occupancy factor based on the root architecture predicted by RootTyp. Once the prototype is stabilised it will be possible to explore differing parametrisations of RootTyp to determine phenotypic impact of changing aspect of root system architecture known to be genetically controlled.

3.4 Transpiration efficiency

In APSIM, daily biomass increment depends on whether the crop is limited more by light or water. In light-limited situations, crop growth depends on radiation intercepted and radiation use efficiency (RUE), and the demand for water as transpiration is determined from the ratio of crop growth to transpiration efficiency (TE), with the latter adjusted for the effect of daytime vapour pressure deficit (Sinclair et al., 1984). In water-limited situations, which occur when the potential supply of water from root uptake cannot meet this transpiration demand, daily crop growth is calculated as the product of the transpiration supply and TE. TE depends on both genetic and environmental components as it incorporates both the intrinsic transpiration efficiency of a species as input to the model and adjustment for VPD:

$$TE = k_d / VPD$$

where VPD is the vapour pressure deficit and k_d is the intrinsic transpiration efficiency coefficient that reflects the equilibrium CO_2 concentration in the stomatal chamber (Sinclair et al., 1984). Hence, TE shows an inverse relationship with the vapour pressure deficit.

To introduce the capacity to moderate TE via hydraulic effects in addition to other effects that likely operate via k_d , a layered canopy photosynthesis model was introduced, and crop growth and potential transpiration calculated throughout the diurnal cycle. Briefly, the canopy model partitions leaf area in each canopy layer into sunlit and shaded fractions, determines direct and diffuse light extinction through the canopy and thus incident light flux for each leaf fraction, calculates photosynthesis from the known light response curve, makes allowance for respiration and biochemical conversion efficiencies, and integrates over the canopy. The model allows for variation in incident direct and diffuse radiation associated with latitude, time of year, time of day, and atmospheric conditions, based on the degree of transmission of extra-terrestrial radiation. The diurnal cycle of radiation input is estimated from readily available daily total and calculated day length. For maize, maximum photosynthetic rate was set at $2.07 \text{ mg CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ and the overall conversion efficiency at $0.41 \text{ mg (biomass) } \{ \text{mg(CO}_2) \}^{-1}$. Potential transpiration through the diurnal cycle could then be calculated from the potential growth rate and ambient VPD, given k_d . This capacity to model crop growth and water use through the diurnal cycle facilitates simulation of transpiration rate limitations that might arise from hydraulic conductance limits.

4. Simulation of trait values measured in panels over European climatic scenarios

Traits have different effects on yields in the different drought or temperature scenarios that occur over Europe. Furthermore, §2 shows that a given allele most often has different effects (from null to high effects) in different environmental scenarios. Hence, breeders have to fine tune the match between a given series of alleles and a given European region. A network of experiments, even a large one such as the networks for maize and durum wheat in DROPS, cannot cover the whole diversity of combinations of temperature x drought x soil depth x all other conditions that affect genotypic effects on yield. Accordingly, we have simulated the comparative advantages of high vs low values of each trait in a series of sites/ years that represent drought and temperature scenarios in Europe. These high vs low values of traits are here the extremes in our panels of genotypes so they correspond to existing combinations of allelic effects. The crop model is the modified version of APSIM described in §3 and the model SIRIUS for durum wheat for which we benefit of an approach similar to that in §2, carried out by P. Martre (INRA, Montpellier) in the frame of companion projects.

4.1 Simulated cultivation techniques

Fifty five sites have been studied across Europe for maize, and 10 for durum wheat. Locations have been chosen according to the cultivated area of each species, the availability of data and, for wheat, a priority to the southern part of Europe where most durum wheat is grown. Climate and soil data have been extracted from the European database of the Joint Research Center for 35 years. Hence, the model was run in 55 sites x 35 years for maize and 10 sites x 35 years for wheat.

Cultivation techniques were simulated in each couple site x year.

- In maize, we have simulated genotypes adapted to each European region. Indeed, farmers choose hybrids with longer cycle duration (in thermal time) over a north-south transect in such a way that crops can photosynthesize for the longest duration as possible. If a common hybrid was grown in north-south transects, this genotype would have excessive cycle duration in the north, resulting in a harvest in bad conditions, or too short cycle in the south, resulting in low yield. The commercialization of maize hybrids by seed providers is therefore organized by groups of precocity. Each group of precocity has shifted to the north in the last 20 years due to global

warming. Based on grey literature, we have simulated these trends by assuming that farmers aim to harvest at a given date regardless of latitude, rather than harvesting at a given thermal time that would result in non-realistic harvest times.

- A common cultivar was simulated for durum wheat, consistent with grey literature. Indeed, wheat has the intrinsic plasticity to adapt its cycle duration via photoperiodic effects.
- Sowing dates were assumed to be common to all sites in wheat, but at a date depending on the frequency of frost at potential sowing dates in maize. Again, this is consistent with grey literature stating farmers' strategies.
- Fields were either rain fed or with full irrigation. We are aware that rain fed agriculture may not be realistic in Southern Europe, but the two treatments were still kept throughout for analysis purpose.
- Other techniques such as fertilization and plant density were kept constant over whole Europe, at values chosen to maximize simulated yields. This has allowed us to focus this study on temperature, evaporative demand and soil water deficit.

We estimated 2050 climate conditions by down-scaling global circulation model data using a proven stochastic weather generator to produce synthetic sequences of daily weather, thereby preserving the historic statistical trends of site data. Climatic conditions for 2050 showed increased temperature throughout Europe, but with greater magnitude in the south, along with cropping season rainfall decrease in France, Italy and Spain, but only marginal change elsewhere. Crop simulations were then repeated using 100 synthetic years representing the 2050 climate but retaining historical site characteristics, with the same model settings, except that transpiration efficiency was increased by 21% to account for increased atmospheric CO₂ concentration.

4.2 Environmental conditions and yield

Soil depth of studied sites ranged from 0.6 to 1.5 m, a reasonable range in European conditions. Maximum air temperature at maize flowering time, averaged over 35 years, ranged from 23 to 31°C. Drops experiments for maize were therefore performed at temperatures higher than 35-year average. Maximum temperatures at durum wheat flowering time were cooler because flowering time occurs earlier than in maize. The degree of water deficit during flowering time in rain fed fields was simulated via the supply/demand ratio. The latter ranges from 1 in conditions in which plants do not suffer any drought to 0.3 in very severe stresses. Water deficit was frequent in southern sites, but appreciable water deficits were also observed in more northern sites. High year to year variabilities were observed in all cases. It is noteworthy that the very severe droughts simulated in Spain and Greece are not realistic for maize, which is irrigated in most cases in these regions.

Maize yields simulated with APSIM ranged from 0.9 to 9.5 t ha⁻¹ in rain fed conditions, and from 7.5 to 12 t ha⁻¹ in well-watered conditions. This exactly corresponds to the range of yields that have been observed in the DROPS networks of experiments, thereby suggesting that the calibration of the model is correct. Highest yields were simulated in the deep soils of Eastern Europe, while sites at higher latitudes had intermediate yields. Yields were in a narrower range in well-watered conditions with best sites in the Po valley.

Durum wheat yields simulated with SIRIUS ranged from 3.6 to 6.3 t ha⁻¹, again in the same range as in the experiments carried out in the network of fields. As expected, maximum yields were observed at highest latitudes. Yields were not simulated in irrigated conditions for durum wheat, a nearly non-existing technique for this species in Europe.

4.3 The effects of climate change on yield may be lower than expected, and will depend on the frequency of stress episodes similar to those observed today

4.3.1 Simulated yield in low stress conditions will not be appreciably affected by climate change in maize provided that farmers adjust their genotypes. In current conditions (last 35 years), around 40% of (rain-fed) fields experience well-watered conditions. This proportion will decrease to 26% in 2050, but with no major effect on yields. In this case, potential yield is determined by the amount of intercepted light during the cycle. Any increase in temperature reduces the duration of the crop cycle and ultimately crop yield. Provided that farmers adapt their genotypes to warmer conditions, we found that the mean yield in 2050 environments with low levels of drought stress was within one standard deviation of that under current climate conditions. Such adaptation by farmers is observed in grey literature, with groups of precocity moving northward with time. It is simulated here by considering that farmers aim to harvest at a given date (§4.2), thereby choosing genotypes with increasing cycle duration in thermal time as temperature increases.

4.3.2 The frequency, not the nature, of the four drought stress patterns will change by 2050. The remaining 60% of cases in current conditions was classified into three categories according to the timing of onset and intensity of water deficit. Early, severe terminal stresses initiated prior to flowering (18% of cases) were the most detrimental for yield, followed by early stressed relieved after flowering (18% of cases), and late terminal stresses initiated at flowering (22% of cases). Clustering climatic years of 2050 resulted in the same drought scenarios as those for the period 1975 – 2010, but with increased frequencies, namely 25% for early terminal deficit, 16 for early stresses relieved after flowering and 32% for late terminal stresses. The yield responses to climatic scenarios and to trait variation were remarkably similar under 2050 climate conditions compared with current climate. We can therefore conclude that current breeding strategies will be relevant for future climates. The main difference is that because the frequencies of water deficit and high temperature will increase in 2050, breeding strategies should be weighted more towards these conditions in the future.

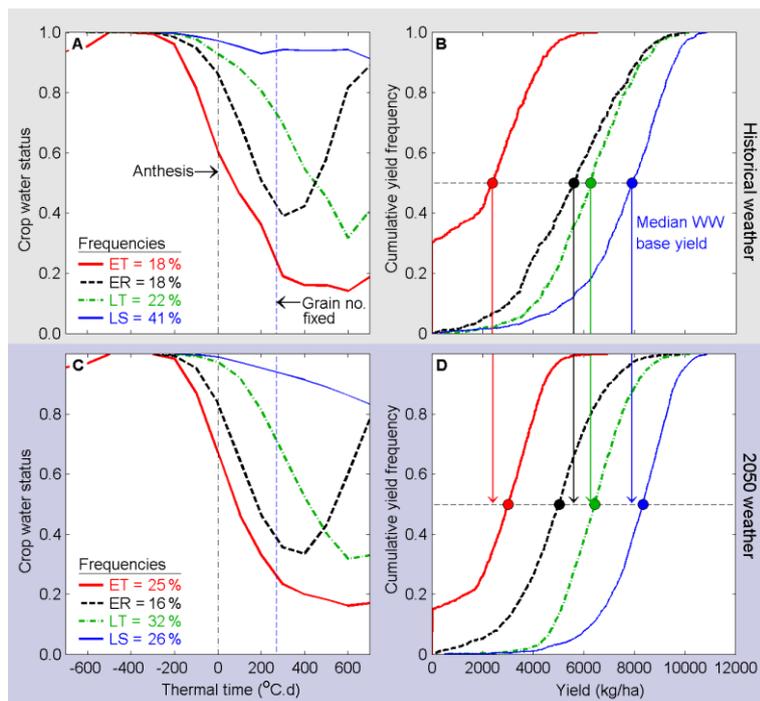


Fig. 16. Crop water status trajectories versus crop development stage expressed as thermal time before or after anthesis for current (a) and 2050 (c) climate conditions for the four drought stress seasonal patterns identified (ET = early terminal, ER = early relieved, LT = late terminal and LS = low stress). Associated frequencies of occurrence are indicated. (b, d) Cumulative simulated yield frequencies across all sites for seasons in each seasonal pattern for current (c) and 2050 (d) climate conditions. Points on curves indicate median yields for each seasonal pattern in each climate scenario. Vertical arrows are included for comparison across climate scenarios.

4.4 Effects of a genetic variability of root growth rate on European yields

For all traits studied below, we have considered trait values corresponding to extreme genotypes of the DROPS panel, measured in phenotyping platforms. Figures represent the difference in yield in each site between the simulated genotypes with highest and lowest values of the considered parameter.

The parameter *root growth rate* corresponds to the rate of progress of rooting depth, *i.e.* of the depth at which an appreciable proportion of soil volumes contain at least one root. For maize, we have used mean values obtained by root mapping on vertical soil trenches (Tardieu & Manichon, 1987), namely 10 mm d⁻¹ from emergence to the stage "7 appeared leaves", then 23 mm d⁻¹ until flowering time. The latter value, observed in the field, is similar to the mean value for the DROPS panel in the phenotyping platform of Louvain La Neuve, where root growth rates in extreme genotypes were 20 and 25 mm d⁻¹. In the same way, durum wheat was simulated with a mean value of 20 mm d⁻¹ with extreme values of 18 and 22 mm d⁻¹.

Root growth rate had an effect on yield that ranged from moderately negative to positive depending on sites and years, from -0.4 to +1.3 t ha⁻¹ in maize and from -0.03 to +0.1 t ha⁻¹ in durum wheat, with a median value close to 0 in both species. Appreciable increases in yield were only observed in fields with soils deeper than 1.5 m, occurring in only 30% of cases in the network of fields simulated here. In these fields, increases in yield by more than 1 t ha⁻¹ were only observed in case of moderate water deficit. In shallower soils until 1.2 m depth, the effect was negligible, with even negative effects in soils with 1-m depth, and variable effects in soils with 1.2 m depths.

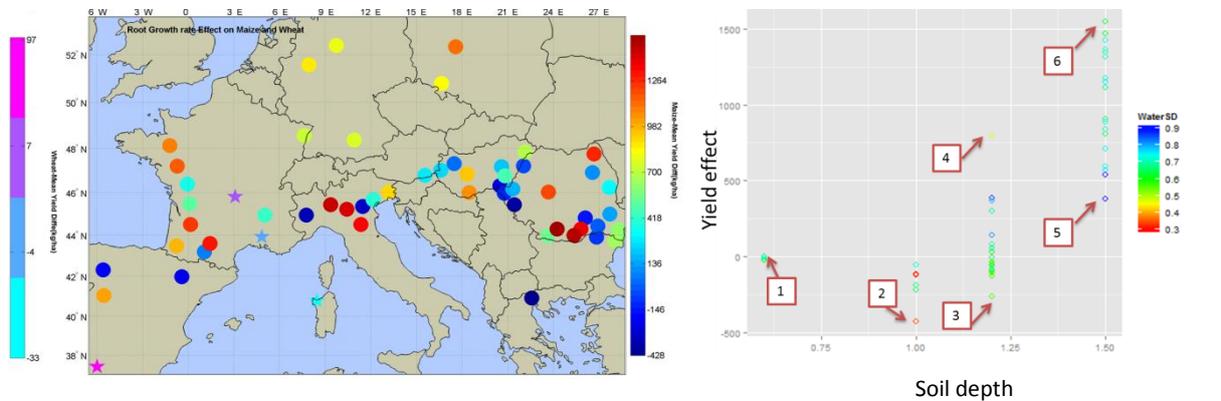


Fig. 17. Effect on yield of root growth rate in maize and durum wheat in each studied site (left panel); circles, maize, stars, durum wheat. Colour represents the difference in yield between virtual genotypes with high and low values of root growth rate. Right panel, difference in yield plotted against soil depth in fields with varying level of water deficit. Blue, well-watered conditions, S/D ratio close to 1, red, water deficit, S/D=0.3).

This variable effect between fields can be explained by the progression of rooting depth in relation to soil depth. Root growth rate had nearly no effect on final rooting depth in shallow soils, so the effect on yield was close to zero (situation 1). In fields with moderately shallow soil (situation 2), there was a genotypic difference in the time course of rooting depth, but no difference in final rooting depth. In water deficit, the effect on yield of a rapid root growth rate was negative because soil was depleted more rapidly in the genotypes with highest root elongation rates, so these genotypes were more stressed than other genotypes at flowering time. In deep soil, rapid root elongation rates had a positive effect, stronger in moderate water deficit than in well-watered conditions (situations 5 and 6). Fields with intermediate soil depths showed either positive or negative effects depending on whether the increased root elongation

rate resulted or not in a difference in final rooting depth. A contrast in final rooting depth only occurred in case of early flowering time linked to high air temperatures (situation 4), which was not the case at cooler temperatures in situation 3. This results in the logical but non-intuitive result that the effect of root elongation rate on yield depended on air temperature, in addition to soil depth and water deficit.

In durum wheat, the effect of root growth rate on yield was negligible in all cases. Unlike the case of maize, the duration of root growth is long from emergence to flowering time, so roots reached soil depth at flowering time in all cases, as in situation 1 of maize. Hence, the effects on yield were either slightly positive or slightly negative but very low in all cases.

Overall, these simulations formalize the intuition that any agronomist may have about the effect of root growth rate on yield. This effect basically depends on soil depth, with negligible or even negative effects of a high root growth rate in a shallow soil, and a positive effect in deep soil only in cases of water deficit. These results suggest that the "steep, cheap and deep roots" ideotype proposed by other authors to achieve drought tolerance (Lynch, 2013) must be contextualized. As any other trait, root growth rate has trades off that depend on environmental conditions and is no 'magic bullet'. The effect of root angles on the horizontal spread of root exploration, whose genetic variability is considered in §2 for wheat and maize, may have a larger effect than rooting depth, although this effect probably depends on environmental conditions as well. However, we need a more detailed model of root system and water extraction for simulating the effect of root angles on plant water uptake.

4.5 Effects of a genetic variability of leaf elongation rate and of its sensitivity to water deficit

We have simulated the effect of two traits over the whole set of sites and years, namely maximum leaf growth rate, representing the ability of the considered genotypes for growing rapidly, and the sensitivity of leaf growth rate to water deficit. In maize, values of maximum growth rate and sensitivity were those of extreme genotypes of the DROPS panel as measured in the PhenoArch platform (Montpellier). The same applied to durum wheat, with an experiment in the Plant Accelerator of Adelaide (Australia).

The results are presented via the difference in yield between (i) virtual genotypes with highest and lowest leaf growth rates in favourable conditions, (ii) virtual genotype with lowest and highest sensitivities. Differences have been expressed in this way for a more intuitive interpretation, because low sensitivity results in higher leaf area in stressing environments. An increased leaf growth rate affected yield with positive or negative effects depending on environmental conditions, from -0.5 to $+0.2$ t ha⁻¹, with a median value close to zero in rain-fed conditions, and was positive in all cases in irrigated fields (not shown). High leaf growth rates had a clearly positive effect on yield in northern sites with no water deficit, and had an increasingly negative effect in more southern latitudes. The same applied to the effect of the sensitivity of leaf growth to water deficit. Most efficient genotypes were those with a low sensitivity (larger leaves) in northern sites, and those with high sensitivity (reduction in leaf area with water deficit) in southern sites. The same applied to durum wheat.

The difference in yield between genotypes with high and low values of maximum leaf elongation rate was closely correlated with the degree of water deficit around flowering time, estimated by S/D. The effect on yield was clearly positive in sites with well-watered conditions (either rain-fed fields in northern Europe or irrigated fields everywhere), in which the genotype with highest leaf growth rate has a maximum light interception from the beginning of the crop cycle, resulting in highest yields. This effect was increasingly negative with more severe water deficit because fast-growing genotypes experienced more severe water deficit at flowering time, resulting in a lower

yield than slow-growing genotypes. The negative correlation between yield effects and degree of water deficit can be explained in the same way, because sensitive genotypes end up with a lower leaf area than non-sensitive ones in southern sites with water deficit.

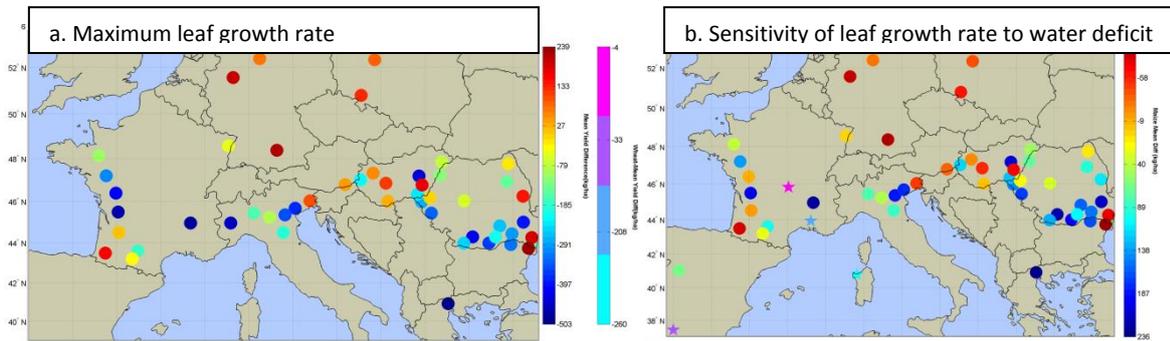


Fig. 18. Effect on yield of leaf growth rate (a) and sensitivity of leaf growth to water deficit (b); circles, maize; stars, durum wheat. Each colour represents a class of difference in yield between virtual genotypes with high and low values of leaf growth rate (a), or between virtual genotypes with low and high sensitivities to water deficit (b).

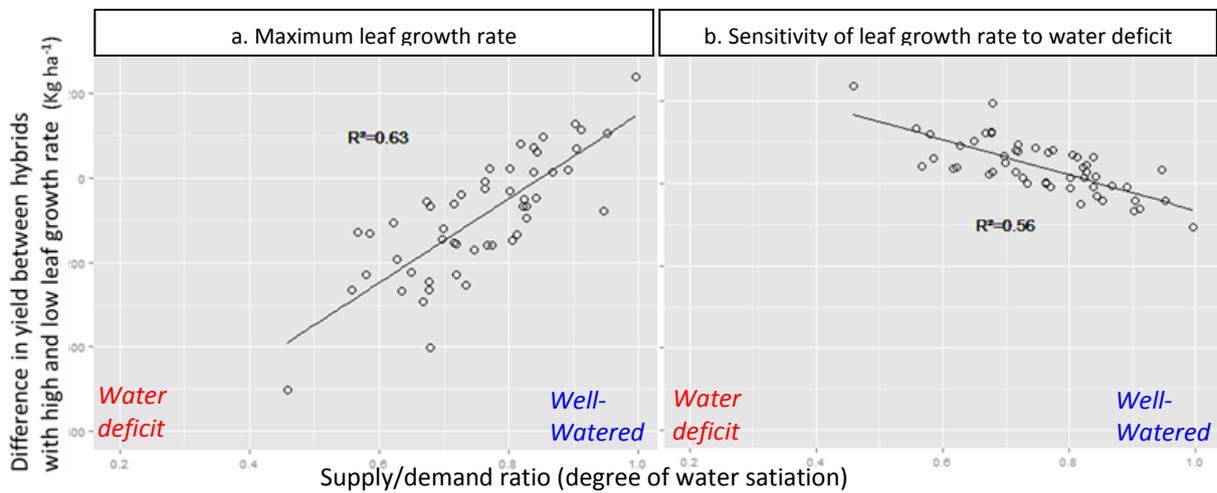


Fig. 19. Difference in yield between virtual genotypes with (a) high and low values of leaf growth rate, (b) low and high values of sensitivity, plotted against the mean supply/ demand ratio in the same fields for 20 days around flowering time (from 0.3, severe stress from 1, well-watered). Each symbol, one coupled value of yield difference and S/D corresponding to one field.

4.6 Effects of a genetic variability of Transpiration Efficiency on European yields

TE has been measured in the phenotyping platforms of Lancaster and Montpellier at several phenological stages. It has a limited but appreciable genetic variability of about 10%. We have simulated the effect of this variability on yield via the difference in yield between virtual genotypes with high and low TE. Simulations predicted that high TE has an effect from 0 to +2.3 t ha⁻¹ in the different sites for the mean of 35 years (+ 3.2 t ha⁻¹ for simulations in individual couples site x year). At low grain yields caused by severe water deficit, transpiration was so low that TE had a small effect on yield. The effect of TE on yield globally decreased with yield and with plant water status. This is because, in APSIM, TE has a greater role when the S/D falls below a threshold, indicating a water deficit. When plants are subject to increasing water deficit (decreasing S/D), biomass accumulation shifts from being limited by radiation use efficiency (the ratio of accumulated biomass to intercepted light, mainly dependent on photosynthesis and

respiration) to being limited by TE. This explains why TE has lesser effect on yield as the S/D ratio increases. A large series of fields showed no effect at all of TE on yield, when S/D remained close to 1 during the whole crop cycle.

4.7 Conclusion of the simulation exercise

We are aware that care must be taken before conclusively stating that the genotypic effects presented here correspond to the reality. However (i) the models have been calibrated, and will continue to be optimized and tested against real yields, (ii) several conclusions presented here correspond to "expert saying" so they can be considered as plausible. The interest of this exercise is to formalize the trades off of traits in a large number of climatic conditions, and to raise hypotheses that can be further tested. It has also an important role for designing genotypes adapted to future climatic conditions as affected by climate change.

Finally, the upper and lower values for extreme genotypic values used in this study can be replaced by trait values calculated from genomic data in hundreds of genotypes, via genomic prediction algorithms that predict the value of each parameter from a linear combination of allelic values. This work will soon begin in the context of companion projects.

5. General conclusion

At the beginning of DROPS, the challenge was to progress jointly in (i) identifying novel mechanisms associated with the response of traits to water deficit, (ii) implementing novel methods of association genetics on these traits and on yield in networks of fields, (iii) developing a modelling approach allowing one to interpret jointly progresses in the last two points, and to simulate the effects on yield of the genetic variability of traits in European regions under current and future climates, and (iv) organising DROPS data in such a way that they allow meta-analyses by DROPS partners, but also by the scientific community consistent with the EC strategy of "open data". We can consider that this challenge has been met.

- Several novel mechanisms are linked to coordination in the plant. This is the case for (i) the coordinated responses of several functions to temperature (§111), that resulted in both a simplification of models and in novel questions in ecology, (ii) the coordination of root elongation rate in different roots of a plant (§132), which also simplify the model and raise questions on the control of root elongation rate, and (iii) to a large extent for the novel mechanism proposed for grain abortion in maize (§121), linked to the superposition of two events namely the sequential emergence of silks originating from ovaries of different cohorts along the ear and the cessation of silk growth that occurs on a single day. At a higher level of organization, several QTLs of yield were superimposed on QTLs of flowering time in durum wheat and maize (§212, 222) in spite of the fact that flowering time was kept in a narrow window. This suggests common mechanisms for phenology and yield.

- Both physiological and genetic arguments plead for a major role of hydraulics in vegetative growth, but also in reproductive development. Leaf growth rate (§112 and silk growth rate (§121) have time courses closely related to those of hydraulic processes, and are not linked to carbon availability. Furthermore, QTLs of responses of growth to water deficit co-localize with eQTLs of water channels PIPs (§213, 214). A novel hydraulic model has been built, then its main features have been translated into the crop model for both leaf and reproductive developments. In bread wheat, the instability of a QTL of yield according to environmental conditions (§321) suggests that the mechanism behind this QTL may well have hydraulic implications.

- Yield QTLs identified in the three species in drought conditions were highly unstable depending on environmental conditions (§212,222, 321), thereby supporting the rationale of DROPS. Precise measurement of environmental conditions resulted in the identification of environmental scenarios in which alleles at QTLs have positive, negative or no effect.
- Another rationale of DROPS was that the genetic variability of root system architecture, rather than root biomass or rooting depth, is a key trait for water uptake. This has been confirmed by simulations in §4.4. A large genetic variability has been found for root architecture in the three species, with interesting QTLs (§213, 223), indicating that genes affecting root architecture will be identified in a near future and can be used in breeding programmes.
- The link between physiological studies, precise models at organ level and crop model has worked for the four traits studied in DROPS. This results in new generation models, currently implemented in APSIM but which can be implemented in modelling solutions involving other modelling frameworks. This has resulted in simulations of the consequences of trait values over Europe, with original conclusions.
- Climate changes will differently affect yields depending on trait values and on the farmer's choice of genotypes. This is a crucial conclusion, which cannot be addressed in current modelling approaches of climate change impacts that are implemented based on a constant genotype until 2050. The modelling effort initiated in DROPS has therefore large consequences for the evaluation of climate change impacts (see § 'societal impacts').
- Finally, an original information system has been developed, which is the base for further projects and for the European ESFRI infrastructure of phenotyping EMPHASIS recently accepted for a preparatory phase in the 2016 ESFRI roadmap. A European information system for phenotypic data is a major issue, to which DROPS has contributed. The information system will also allow public release of DROPS data, consistent with the "open data" strategy of EU.

Impact

1. DROPS contributes to mitigate the impacts of climate change on European agriculture

1.1 Novel simulations show that the impacts of climate changes on yield can be counteracted by farmer's choice of genotypes

Simulations of the impacts of climate changes predict drastic decreases in yields by 2050, based on the extrapolation of the behaviour of a standard genotype per species to future environmental scenarios. This can be either via statistical modelling, based on regression models of yield to environmental conditions, or via crop simulation models parametrized with standard genotypes.

1.1.1 DROPS has adopted a simulation approach based on farmer's practices and environmental scenarios, rather than unique prediction option for whole Europe.

a. Farmer's choice of genotypes is dynamic, based on the opposition between risk and expectation of high yields. In spring/summer crops such as maize or durum wheat, farmers constantly adapt genotypes by 'tries and errors', especially the duration of the crop cycle. This duration has a large genetic variability, in such a way that farmers can choose genotypes with a duration adapted to their climate but also their strategy to deal with risks. Choosing a genotype

with long cycle duration maximises potential yield, but also the risk of harvesting in bad conditions (autumn rainfalls) and of terminal water stresses. This is, therefore, a risky strategy that maximises potential yield. Conversely, choosing a genotype with short cycle duration minimises the risk but also the potential yield. More than 100 new genotypes are registered every year for most crops in Europe and are provided with indications of their cycle duration. A typical time-life of a commercial genotype is five years, so *it is highly unrealistic to predict yields in 2050 based on current genotypes.*

With climate change, the growing area of genotypes having a given cycle duration slowly moves every year in a northern direction. Genotypes with cycle duration adapted to latitudes of 43° (e.g. Pau or Bologna) twenty years ago are now typically grown at latitudes of 46° (e.g. Poitiers or Budapest). This is because farmers in a given site have adopted genotypes with increasingly long duration in order to counteract the increase in temperature that would shorten cycle duration otherwise. *Farmers are, therefore, already adapting genotypes to climate change.*

The strategy followed in DROPS simulations has been to consider that farmer will continue adapting genotypes in order to harvest at a time unchanged in relation to current practices, and to adopt a sowing date slightly earlier than today due to lower frequency of frosts. This tends to reduce the impact of global warming, which decreases yield simulated by current models via shorter cycle duration.

b. Farmers already take into account the risks of abiotic stresses when choosing genotypes. Registered genotypes are increasingly described with phenotypic characteristics, in addition to cycle duration. Farmers are used to take these characteristics into account when adopting genotypes. This intuitive approach will be increasingly assisted by the simulation of risks in every site, based on environmental scenarios as those presented in §43 of the 'result' section. This strategy is followed by several seed companies such as Monsanto, Syngenta and Limagrain, who sell packages to farmers, consisting in risk studies, seeds of adapted genotypes and techniques to counteract unexpected climatic events. *It is therefore reasonable to assume that genotypes chosen in a given region will optimise yield expectations and risk avoidance.*

The strategy followed in DROPS simulations has been to simulate the choice of genotypes assuming that farmers will optimise these choices, based on the simulations of allelic effects proposed in § 4.3 of "results".

1.1.2 A lower impact of climate change on yield predicted by DROPS than currently predicted in most models used by IPCC.

Simulations based on the hypothesis of an adaptation of crop cycle to climate change result in the unexpected result that, in well-watered conditions, climate change will have a limited impact on yield. This result has been published in Global Change Biology (impact factor 8) and has received a large interest in several congresses.

We are currently performing simulations of the impact of climate change under the hypothesis that farmers will counteract the increased risk of heat and water stress by choosing adapted genotypes. Current results strongly suggest that this second source of adaptation also contribute to a lower impact of climate change.

1.1.3. Societal impacts of this change in view

We believe that this change in view, which considers farmer's practices, genetic progress and climate simulation, will have a large impact on several European institutions or economic sectors (i) European agriculture may stay a potent economic sector in spite of climate change, provided

that the offer for improved traits continues in the future (ii) the strategies of European organisms of seed certification should maintain a large diversity of genotypic characteristics in terms of traits and cycle duration, rather than focusing on maximum yield only, (iii) there is large room for SMEs focused on advice to farmers based on local environmental measurements, such as *The Climate Corporation* in the USA recently bought by Monsanto.

1.2 DROPS provides the scientific community with a novel way to identify sources of genetic progress under climate change

1.2.1 Combining genetics, precise environmental measurements and modelling allows identifying in 2016 genomic regions that will counteract the effects of climate change in 2050.

The close association of agronomists, geneticists, statisticians and modellers results in the identification and in the modelling of the effects of a series of genomic regions associated with increased yield or to traits associated to it in dry or hot conditions. Most breeding programmes face the difficulty of instability of the effects of genomic regions associated with plant performance under stress. Indeed, these genomic regions most often have opposite effects depending on the timing and severity of stresses. This instability has been confirmed by DROPS, which has also opened the way for dealing with this difficulty.

DROPS has modelled allelic effects depending on environmental conditions (see §2 in the 'result' section). In particular, alleles at some of identified QTLs have an effect on yield that clearly depends on either soil water status, or evaporative demand or temperature, resulting in contrasting effects in each environmental scenario. Modelling allelic effects allows extrapolating the effects of these alleles in future conditions, thereby considerably improving the capacities of genomic selection. This area of research is currently continued in several ongoing projects, e.g. ANR Amaizing and in H2020 proposals. It is noteworthy that this approach requires few investments in addition to the cost of classical networks of field experiments. Indeed, weather stations with good accuracy and temporal definition cost a few hundreds of Euros compared with the tens thousands Euros associated with current experimental procedures.

The proof of concept by DROPS of a novel way to analyse networks of field experiments has been used in further projects (e.g. ANR-PIA Amaizing, 2012-2019), and has resulted in three of the four 'pillars' of the European infrastructure of phenotyping (EMPHASIS) recently accepted in the ESFRI 2016 road map, namely highly equipped phenotyping platforms in controlled conditions, networks of field experiments and model-assisted data analysis.

1.2.2 Via its "open data" strategy, DROPS provides a large and consistent dataset that can be analysed by several groups interested in climate change now and in the future.

DROPS has dedicated a whole workpackage to the building of an information system that organizes and stores environmental and phenotypic datasets, both in field and in controlled platforms, in the three studied species. Re-analysing data with different points of views and hypotheses will be a crucial activity of many groups in the future, in view of the cost for obtaining a dataset including full environmental conditions, yield data in large networks of fields, detailed phenotypic data in robotized phenotyping platforms and omic data such as transcripts and metabolites in the same genotypes studied in the field and in platforms. We have therefore considered as an essential activity to organize this dataset with sufficient rigour and annotations in such a way that other groups can benefit of this dataset. This may be the case for groups wishing to test alternative methods or hypotheses in the next years, but also in the future to compare the effects of alleles or genotypes in environmental conditions prevailing in 2016 to

those tested in tens of years. We think that this effort of DROPS will result in a long-term effect because the datasets corresponding to each species will be the base of future research requiring minimum additional investment. Data are already organized and will be made available on demand after publication.

This workpackage has developed novel methods for connecting datasets obtained in different platforms and for centralizing field datasets. *The approach of information systems developed by DROPS is one of the bases for a European phenotypic information system, to be built in the ESFRI project EMPHASIS.*

2. Impact of DROPS on breeding activities and seed companies

2.1 Novel methods have been adopted by the four European breeding companies partners of DROPS

2.1.1 Partner breeding companies have massively adopted the environmental characterisation diffused by DROPS.

The four European breeding companies that are DROPS partners (Limagrain, Syngenta, KWS, Biogemma) have benefited of the proof of concepts performed during the project. As a result, it is striking to note that most field experiments performed by these companies now include networks of weather stations and sensors. For instance, Limagrain has launched programmes including environmental measurements in the field and the development of statistical environmental indices in a PhD thesis (Bouffier et al 2014).

2.1.2 Modelling is increasingly used by partner breeding companies

Pioneer-Du Pont, a DROPS partner for modelling activities, has based its breeding strategy on simulations based on a crop model. This company was interested to participate to DROPS without EU funding and to share the developments of a crop model in which they have massively invested. This has resulted in publicly available module for traits developed in DROPS.

The DROPS proof of concept of the use of models in genetic activities and the example of Pioneer-Du Pont has contributed to an evolution of European seed companies towards the use of crop models. This is part of their internal strategy and cannot be diffused here. However, DROPS is proud to have contributed to this change in view of European seed companies.

2.1.3 Genomic regions improving yield in dry or hot conditions have been included in the breeding programmes of partner seed companies, and will be further tested in companion projects.

Limagrain, Syngenta and KWS have included the genomic regions identified by DROPS in their breeding programmes, either directly or via genomic prediction. These breeding programmes jointly evaluate the effects of hundreds of markers, and are secret for obvious reasons. Hence, we will not be able to identify the contribution of each genomic region in the development of novel genotypes.

The same three companies, plus Biogemma, will be involved in the test of the effects of these genomic regions in lines carrying introgressed regions, together with public groups, in further field and platform experiments. This will be performed in the context of companion projects in particular ANR Amaizing (2012-2019).

2.2 Diffusion to the breeding community (in particular breeding SMEs) via a conference organized with the European breeder association Eucarpia.

Diffusion to non-partner scientists involved in breeding, public or private, has involved the European breeder association EUCARPIA. Jointly with Eucarpia, we have organized the conference “Recent progress in drought tolerance: from genetics to modelling” on 8- 9 June,

2015 in Montpellier, France, coupled with a training session on phenotyping on 12 June. The aim was to provide an accurate view on recent advances in the mechanisms associated with plant response to water deficit, on their phenotyping, on their genetic variability and on the modelling of relevant allelic effects on plant behavior under changing climates. The scientific committee of the conference was the executive committee of DROPS. The organization committee, chaired by Dr Claude Welcker, involved INRA, UNIBO, Inra Transfert and Eucarpia.

This conference reached an audience of 305 scientists, i.e. a similar number compared with the last Interdrought conference in Perth. This placed this conference as a major event in the domain of drought. In total, 136 scientists presented their works, 15 were invited speakers (11 from the DROPS consortium), 10 were selected speakers, 30 were young scientists with 2-min talks presenting their posters. A "forum" on breeding consisted in a dialogue between the audience and a panel of 5 renowned breeders.

A survey indicated that the audience involved 57 scientists from the seeds industry and 28 from international research organizations dedicated to breeding. 232 scientists came from developed countries, 73 from Mediterranean (27), African (14), Middle East and Asian (19) and South American (13) countries. Among those, 14 have received financial support with the help of the Montpellier Agropolis Foundation. In addition to the sum allocated to this meeting in the DROPS budget, additional funding was provided by INRA and the region Languedoc Roussillon

The programme was as follows:

Session 1: Coping with drought in crop improvement: a global perspective

Session 2: Traits-base strategies for dealing with drought

Session 3: Variation for target traits and phenotyping methods

Session 4: Dealing with Genotype x Environment interaction

Session 5: QTL, functional genomics, allelic diversity and breeding application

Session 6: Breeding for drought, strategies, choices

Panel discussion with renown breeders: Designing new variety for drought-prone environment

A technical session "Phenotyping for the responses to environmental conditions" was organized on June 12th. It consisted of presentations of the DROPS approach of phenotyping and in presentation by and Australian colleague. Participants visited followed demonstrations of the sensors and imaging systems in platforms and in the field via UAVs.

3. Contribution to knowledge-based economy by publications and training students, post doc and breeders for a multi - disciplinary approach

3.1.1 DROPS has resulted in 52 publication in scientific journals, and has been presented in 149 international conferences, either for its strategy or for striking results

The strategy of DROPS has been presented as such in five invited presentations in international conferences dedicated to either drought or to strategy in Plant Science, in addition to the conference organized by DROPS in Montpellier. Organizers were interested by a presentation of how DROPS combined different disciplines, the difficulties associated to this approach and its main successes. These were (i) the Interdrought conference at Perth (Australia, 2-6 Sept. 2013), (ii) the XV Latin American meeting of Plant Physiology in Mar del Plata (Argentina 25-28 Sept 2014), (iii) the Climate Smart Agriculture conference organized at Montpellier (France, 16-18 March 2015), (vi) the Stress Resilience Symposium organized by Society for Experimental Botany and Global Plant Council in Iguazu (Brazil, 23-25 October 2015) and (v) the final European Plant Phenotyping Network conference organized in Barcelona (Spain, 12-13 November 2015).

DROPS results have been the object of 45 invited presentations in international conferences.

DROPS has resulted until now in 52 papers, 23 of which published in high ranking journals with impact factors higher than five. Most of those involve multidisciplinary approaches and show the interest of combining either field and platform experiments, or phenotyping plus modelling for drought tolerance. Many manuscripts are in preparation or submitted. A publication plan involves 65 projects of publications.

3.1.2 18 PhD students, 67 post doc and 10 permanent young breeders have been trained during DROPS for translating results from the laboratory to the field

Drops has involved PhD students and post docs and young breeders of seed companies, paid by the project or by other projects, who have been in contact with the DROPS community in annual meetings and have had the occasion to present and discuss their work during open sessions with large space for discussion. They have also had access to the courses organized by DROPS, and in most cases have travelled to other partner's sites to perform joint experiments.

3.1.3 DROPS has organized five "hands on" courses with about 30-40 participants each,

Five "hands-on" courses were organized for young scientists and breeders either involved in DROPS or out of the consortium. Conferences on principles, practical sessions and computer sessions were organised in each course.

Course on Phenotyping (31st January - 2nd February 2011, Louvain-la-Neuve, Belgium). Two topics were presented, namely environmental characterization (air and organ temperatures, evaporative demand, potential biomass production, water transfer, thermal time) and trait analysis (root architecture, leaf growth, water use efficiency, grain abortion, carbon and oxygen stable isotope). It resulted in the creation of a minimum dataset that has then been published as such in an academic journal.

- *Course on Association Mapping* (29-30 March 2012, Istanbul, Turkey). Design of experiments, concepts and models of association mapping, lessons from previous experiences, computer exercises of Association Mapping.

- *Course on crop modelling* (28 March 2013, Versailles, France. Concepts, current issues, examples using the APSIM model.

- *Use of the DROPS database for phenotyping data and for bioinformatics* (28th March 2014, Aachen, Germany). Presentation and computer exercise on the database dedicated to field data. Presentation and computer exercise for the identification of candidate genes behind QTLs

- *Second course on phenotyping and modelling for drought tolerance* (4th March 2015, Bologna, Italy), largely open to students, PhD and post docs of the University of Bologna. Canopy temperature and its use in phenotyping, novel imaging techniques in the field, responses to temperature and novel approaches of thermal time, computer exercises on the APSIM model.

3.1.4 These courses are now available on the DROPS website.

The courses materials have been made available on the former project website (<http://www.dropsproject.eu/>) in the dedicated area 'Training courses'. For ensuring website functioning and survival after the end of the project, a migration has been operated to the INRA server <http://www6.inra.fr/dropsproject>. Website and training courses are now publicly available.



The screenshot shows the DROPS website interface. At the top, there is a navigation bar with links for 'Home page', 'Project overview', 'Consortium', 'Results', 'Online resources', and 'Training courses'. A search bar is located on the right. Below the navigation bar is a banner image of hands holding grain. The main content area is divided into several sections: 'Training courses' with a list of topics like 'Air and organ temperatures', 'Evaporative demande', etc.; 'Environment' featuring an 'Environnement Computer' section with a PDF link for 'Leaf water potential measured with a pressure chamber'; and 'Environnement Exercices' with links to 'Exercice plant development', 'Exercice Micrometeorology', and 'Exercice thermal time'. A 'News & Events' sidebar is also visible. The footer contains copyright information for INRA 2015 and a website URL.

3.1.5 DROPS has also organized the diffusion to the general public, in particular via a 'success story' published in EU website

DROPS has also been selected for a "success story" published in the website of EC Research and Innovation, http://ec.europa.eu/research/infocentre/article_en.cfm?artid=36579 'The DNA of drought tolerant crops'.

It has also been the object of several TV programmes in the French television and of technical papers in Agricultural journals.

4. Contribution to phenotyping and phenotype-to-gene approaches in the wider European community

DROPS has been the first EU project using automated phenotyping platforms in a coordinated way for characterizing different specific traits at high throughput. The same panels of maize, durum wheat and bread wheat were used in several platforms, namely at UC Louvain (Belgium) for root traits (growth rate, architecture, ramification), at INRA Montpellier (France) for shoot traits and transpiration (growth rate, architecture, transpiration, radiation use efficiency and water use efficiency), at Adelaide (Australia) for shoot traits and transpiration (growth rate, transpiration, water use efficiency) and at Lancaster (water use efficiency). Using these platforms at high throughput with common genetic material, together with omic data, has resulted in original genetic analyses, considered as a proof of concept in the phenotyping community.

DROPS has also designed methods for a better interpretation of networks of field experiments by using inexpensive equipment for environmental measurements. The first pieces of equipment for field phenotyping have been tested (e.g. infrared thermometry, radar or images taken from UAVs) but were not mature enough to be used at high throughput for the phenotyping of panels.

Methods for genetic analyses of multi-traits, multi-site experiments have been developed and are now available after publication. This has required the development of novel mixed models which can accept the high number of markers (up to one million), of genotypes (up to 300) and traits used in DROPS. This has required considerable methodological work, which can now be used by a wide community.

The experience acquired during DROPS has been essential for the development of the European phenotyping community, in particular for three of the four "pillars" of the European infrastructure of phenotyping (EMPHASIS) recently accepted in the ESFRI 2016 road map, namely robotized phenotyping platforms (European platforms in DROPS are part of EMPHASIS), networks of field experiments and plant models. The approach of information systems developed by DROPS is a basis for EMPHASIS. All these method and information systems are also an important starting point for the I3 project EPPN2020 solicited by the European commission in the call of 13 October 2015 (common coordinator).