

# 'White paper' on Plant Phenotyping

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## Key Messages

- Plant phenotyping has become a bottleneck for progress in basic plant science and plant breeding
- Europe has world-leading groups in plant phenotyping
- Phenotyping requires interdisciplinary approaches of plant scientists, (bio-)informatics, sensor development and environmental sciences and simulation.
- Phenotyping needs to integrate activities in sensorics, environmental simulation, mechanistic, high-throughput and field-based platforms
- Next steps to implement phenotyping in the European Research Area are the formation of a network of researchers, breeders and industry (European Plant Phenotyping Initiative (EPPI) and of a major European infrastructure network of plant phenotyping platforms.

## Background

Phenotypic analysis has become a major limiting factor in genetic and physiological analyses, in plant sciences as well as in plant breeding. Through rapid developments in plant molecular biology and in molecular-based breeding techniques,

- (i) An increasing number of species have been sequenced and large collections of mutants, accessions and recombinant lines now allow analysis of the gene functions.
- (ii) High-definition genotyping can yet be carried out on thousands of plants in a robotized way, thereby allowing association genetics or multi-parental QTLs.
- (iii) For transcriptomic, proteomic and metabolomic analyses, large robotized platforms are available so the biochemical status of plants can be investigated in detail at reasonable cost.

In contrast, the understanding of the link between genotype and phenotype has progressed more slowly, in spite of recent advances. Faster progress is currently hampered by insufficient capacity (both technical and conceptual) of the plant scientific community to analyse the existing genetic resources for its interaction with the environment. Advances in phenotyping are therefore a key factor for success in modern breeding as well as for basic plant research.

The gap between genes and phenotypes is particularly large in analyses of plant-environment interactions, urgently needed for research and application on sustainable and resource-efficient crop production in the context of climate change and changing agricultural production conditions. Novel genetic methods have mostly been tested on relatively simple phenotypes such as flowering time, which do not require sophisticated phenotypic approaches. The use of the same genetic methods in analyses of tolerance to biotic and abiotic stresses requires the analysis of defined genetic material in multiple well-characterised environmental scenarios. Key plant functions, like cell division, expansive growth, gas exchange, water and mineral status/transport and the role of plant architecture at the above- and belowground levels need to be quantified during the dynamic responses of plants to the environmental conditions throughout their development. Innovative technologies and novel approaches - often based on non- or minimal-invasive techniques – become increasingly available. These allow quantitative analysis of the spatially and temporally developing processes and structures at a mechanistic level, providing the basis for understanding the dynamic interactions between genetic and biochemical processes with physiological phenotypes. They also potentially allow genetic analyses of structural and functional traits at high throughput, not achievable until recently.

However, the analogy of phenotyping and genotyping is somewhat misleading. Phenotyping is, by nature, a process which requires choices in variables, sampled organs and times of sampling, whose relevance depends on the addressed biological questions. (i) Phenotypic variables are multi-dimensional, with time constants ranging from seconds (gas exchange) to several days (cell cycle, plant architecture). (ii) The phenotype originates from the dynamic interaction between the genome, the fluctuating and heterogeneous environment and the multiple plant responses that the plant develops to face environmental cues. (iii) Many phenotypic traits accumulate during the development of the plant, making the response of a plant dependent of previous phenotypic responses.

***Hence, phenotyping is not conceivable without the development of new methods involving novel sensor technologies, databases, statistics as well as plant-environment modelling, able to relevantly analyse the deluge of information originating from genotypes, transcriptomics, proteomics, metabolomics, fluxomics and phenotypic analyses.***

### **Status of Phenotyping**

Several groups in Europe and Australia have worked on the challenge of new methods for phenotyping. European projects in the FP6 and FP7 have identified work packages on the development of new phenotyping methods and on their integration with omics (e.g. FP6 Agron-Omics, FP7 Spicy and Drops). At the European member state level a number of initiatives have been established (e.g. Jülich Plant Phenotyping Centre (JPPC, Germany), Phenopsis and Phenodyn (INRA, France) and form the nuclei for larger-scale activities. These activities address specific topics that are part of the phenotyping process and their integration into a full phenotyping pipeline.

1- "Sensorics", i.e. the development of new technologies that can be implemented into phenotyping methods. These are either adapted from other disciplines like medicine or satellite sensing, or they are developed specifically for the plant application. These technologies often provide images (e.g. multi-spectral, infrared, etc) allowing the analysis of spatial heterogeneity and thus addressing the inherent local and modular organisation of plants. Further developments include tomographic methods (e.g. x-ray computer tomography (CT), magnetic resonance imaging (MRI) or positron emission tomography (PET) that literally provide insight into plant organs and non-invasive analysis of belowground structures and functions and thus the basis for in-depth phenotyping. The development of environmental sensors with good spatial definition and accuracy allow monitoring of the relevant environmental conditions.

2- "Environment simulation facilities" that impose tightly controlled spatially and temporally (either stable or fluctuating) environmental conditions are central for phenotyping designs with predictive value for field performance. Such simulation systems are also necessary to design experiments addressing the capacity of plants to acquire and utilise resources such as nutrients, water or light that are by nature not homogeneously distributed or only available in temporally and distinct patterns.

3- High throughput phenotyping platforms in controlled conditions, able to deal with several hundred up to several thousand of plants in a single run in controlled or tightly monitored environmental scenarios and allowing high precision phenotypic measurements. These platforms are most often dedicated to a limited number of applications, compatible with their technical choices, namely phenotypic target (e.g. plant architecture, photosynthesis, vegetative growth), species (plant size/architecture, monocots vs. dicots...) and type of environmental cues (biotic, water, nutrients). They are, therefore, associated with a series of biological questions. However, approaches for larger and integrated consortia addressing mechanistic, high- and medium throughput as well as field phenotyping are on their way – especially in Europe.

4- Field phenotyping platforms able to follow growth, gas exchange and status of canopies with large number of plants and genotypes, by using proxy detection (imaging or vegetation indices) with sensors placed on tractors, robots or flying platforms. These are combined with accurate environmental monitoring and can be linked to devices able to manipulate environmental conditions (e.g. rain out shelters, CO<sub>2</sub> enrichment (FACE), heating devices, etc...).

5- Methods to analyse phenotyping data and handling their upscaling. The phenotyping platforms provide large datasets, which only reach their goal if they are analysed properly and if data at organ or plant levels (usually in controlled platforms) can be integrated or scaled up to the field with adequate methods. (i) Methods have been made available to mathematically describe traits, which vary rapidly with environmental conditions (e.g. growth or gas exchange) based on model-assisted phenotyping and meta-analyses of large datasets; (ii) the multiplicity of stressful environments, difficult to handle experimentally, can now be addressed due to the progress of models. If a model adequately describes the effects of the genetic variability in a few climatic scenarios, it can then be extended to a much larger number of scenarios in order to evaluate the comparative advantage of a given allele in different environments; (iii) methods for statistical analysis of Genotype x Environment x Management interaction have also progressed enormously, thereby allowing relevant analyses of large networks of field experiments.

### **Critical analysis of the current situation**

- Private seed companies are willing to progress in the 5 items presented above. Major companies have developed their own platforms in the field and in controlled conditions; other European-based companies and SMEs have built public private-partnerships. The approaches taken in these projects or the design of the infrastructures differ with respect to their throughput, tens to hundreds to adequately characterise specific mechanisms of major species or genotypes, hundreds to thousands for the genetic analyses and tens of thousands in the breeding process. It is obvious that a portfolio of methods is required to address these different scales. At least for the next years, public-private cooperation has probably to focus on the first two scales.

- In the public sector there is a risk of a naive approach of phenotyping, with anarchical development of phenotyping platforms which may well result in disappointing results as often methodological and conceptual issues are not properly addressed. This is, in particular, the adequate development of sensorics and environment simulation or monitoring facilities, the identification of limitations of the available platforms in terms of scope and possibilities, and the development of data-handling involving database interfacing, statistics and modelling. Furthermore, groups which first stated the necessity of high throughput phenotyping were not always those which had access to the required combination of measurements, modelling and whole plant physiology. There is, therefore, an urgent need for EU level coordination which will prepare a second generation of phenotyping initiatives and infrastructures, necessary to avoid waste of money, frustration of researchers and irrelevant results. Three crucial needs have to be met, (i) the identification of the most essential objectives in phenotyping in terms of biological questions, sensors and methods, (ii) the development and implementation of innovative phenotyping concepts that address the identified objectives, (iii) the development of new methods, new sensors and new integrated platforms which aim to meet these missing links in a most goal-oriented and cost-efficient way. A risk of 'non applicable applied research' exists if these three steps are not managed jointly.

### **Proposed next steps**

The plant science community is challenged by the large demands described above and by the limited possibilities offered by EU and member states. Private funding can partly be used, but holds the risk that important developments do not become publicly available. The example of the development of a mouse phenotyping coordinated action is a best practice example to follow, keeping in mind that the equivalent in plants would be 'mouse + cattle + Drosophila phenotyping', i.e. dealing with different families of species having considerable differences in genomes, habits, metabolism, size and dedicated use, with plants having much higher plasticity in acclimation to abiotic and biotic impact than animals.

European public and private research must invest in the topic of phenotyping to keep its technological advance, in particular in relation with Australian partners on one hand, and with major seed companies and an upcoming phenotyping community in the US on the other hand. This is of vital interest for public research as well as for the development of Europe-based seed companies and for the emergence of SMEs in the field of biotech. Europe should use its unusual competitive advantage.

We propose three steps:

1. A short-term networking of a European Plant Phenotyping Initiative (EPPI) via a flexible, but professional organisation with a website which mirrors existing activities, workshops, idea and personnel exchange and education and conceptual activities. This is of crucial importance in view of the large diffusion of the Australian initiatives, much more visible than European ones although the state of the art is at best similar. Little, but professional manpower would be needed to develop such initiative, to interface databases and to perform communication actions.

2. A major EU research infrastructure network in FP7 may be launched in a next future, as presented by the EC officer in the workshop. Its main tasks may be to coordinate and facilitate the development of methods carried out in several EU countries. The objective of the initiative would be to develop new methodologies by sharing methods and approaches, taking advantage of the technical progress in other disciplines and of methodological development in plant physiology. It is essential that this group addresses from the very beginning on the questions of the transition from platforms to fields, and the connection with breeding programmes.

The consortium would ideally be formed of two circles, an 'inner circle' of groups who will spend time and energy in developing methods and specific phenotyping infrastructures (sensors, methods, platforms), thereby accepting a risk of slow developments and of errors, and an 'outer circle' formed by groups who would express their priorities, use and evaluate new methods and technologies when they are ready, and finally diffuse these technologies via publications, access to methods and infrastructures of the inner circle and through education.

3. Possibly fund new platforms, equipment and federate the effort of several EU countries in this aspect. There is no doubt that a considerable effort will be necessary in terms of equipment, but also in the support of existing infrastructure which will become an increasing problem. We therefore propose that a major scientific equipment is designed, distributed to a network of a few locations in Europe, which can be proposed to ESFRI in 2010. This design should involve national programmes, national research agencies and leading European groups federated in the network of excellence presented above.