Fertile phenotypes

Phenotyping Lead at the Scientific Affairs Department of the ARVALIS Plant Institute, **Dr Katia Beauchêne**, explains how a French collaboration is making vital contributions to the emerging field of plant phenomics

Is there value to be derived from applying phenotyping to crops?

Nitrogen is the most important inorganic nutrient for crops and is of paramount economic importance since it is also the most expensive. In the 'Development and utilisation of new generation phenotyping tools to analyse genetic determinants of nitrogen fertilisers use efficiency in bread wheat' (PHENOBLE) project, we are testing various novel phenotyping methods to evaluate new traits and gain a better understanding of wheat's nitrogen use efficiency (NUE). We also hope to see how wheat yield components and grain protein concentration vary depending on the nitrogen fertiliser level. Our main aim is to obtain associations between molecular markers and agronomic traits for new phenotypes.

Why is high-throughput phenotyping of wheat a major priority at present?

Wheat yields have stagnated since the mid-1990s. This stagnation has been mainly attributed to an increase in unfavourable growing conditions, such as drought during the stem elongation period and high temperatures during the grain filling period, as well as changes in agricultural practices that have reduced nitrogen availability to wheat crops.

Although our increasing capacity to develop molecular markers on each genotype is quite high, our ability to describe plants in field conditions remains a challenge because it is destructive, labour-intensive and expensive. To improve our knowledge on the physiological and genetic basis of NUE, efforts need to be made in phenotyping to advance our knowledge on both existing diversity and its underlying genetic control. New phenotyping tools currently exist, but only in controlled greenhouse conditions and on small field trials of 10 plots. PHENOBLE aims to transfer these tools to 1,000 plot field trials in order to characterise genotypes for genetic association studies.

How are you evaluating new high-throughput phenotyping methods in- and outside the field and assessing their relevance to plant genetic studies?

We first evaluate new methods by measuring their capacity to be used directly in the field where factors such as weather, wear and tear, and manpower come into play. Secondly, each trait measured by these new methods is evaluated next to those characterised by more 'conventional' measures. Finally, each trait, whether new or conventional, will be examined using genetic association to identify the chromosomal regions involved.

Are any particular tools and technologies having a significant impact on your work?

High-throughput metabolomic tools allow us to characterise the contents of leaves, roots and other plant organs in great detail, and this information is less integrative than complex agronomics traits; but they still require destructive, time-consuming sampling. Conversely, the tools that are non-destructive have the biggest impact on our work because they allow a new dynamic approach. With these kinds of systems (Arche system and ASD Labspec®), we have a kinetic view of each plot, which enables us to understand what happens during its whole development.

How do the PHENOME and BREEDWHEAT projects complement PHENOBLE's work?

Initiated in 2012, BREEDWHEAT aims to combine high-throughput genotyping and phenotyping methods to perform association studies and identify markers and candidate genes for yield and quality traits under major biotic and abiotic stress. As it is also concerned with NUE, it uses PHENOBLE's results to increase the knowledge in field trial networks.

PHENOME, which began in 2013, aims to build a French plant phenomics network of nine platforms, three high-throughput field platforms, two field platforms with control of CO_2 (Free Air CO_2 Enrichment system) and drought (rain-out shelters), two platforms in controlled conditions and two -omics platforms in the laboratory. Using the structure and sensors of the semiautomatic monitoring system developed in PHENOBLE, an automatic high-throughput system called 'PhénoMobile' will be built to equip the field platforms.

What efforts have you undertaken with farmers and breeders to ensure an optimal transfer of knowledge?

All of these results will contribute to the improvement of wheat breeding in the near future; in particular by helping to create cultivars that are better adapted to changing climatic conditions and to more accurately recommend the best choice to the farmers. Furthermore, through complementary projects like BREEDWHEAT and PHENOME, the phenotyping tools validated in this project will be made accessible to the wheat breeding community.

Reaping the **benefits**

Agricultural industries are in need of innovative methods to improve crop yields as the global demand for wheat increases. The **PHENOBLE** project is advancing the field of plant phenomics to address this concern

ACCORDING TO 'A Workshop Report on Wheat Genome Sequencing' by the International Genome Research on Wheat Consortium, the total global output of grain needs to accelerate by 2 per cent each year in order to meet human demands by 2050. Wheat is fundamental to the diet of most of the world's population and is cited by the United States Department of Agriculture as the leading source of vegetable protein. In order to increase output, it is necessary to develop new cultivars that can thrive under significant biotic (pests) and abiotic (drought, frost, salinity) stressors. Of particular importance to environmental conservation and the agricultural industry alike is wheat's ability to process nitrogen. As modern wheat cultivars require high nitrogen fertiliser inputs to sustain high yields, optimised nitrogen use efficiency (NUE) would be extremely beneficial to agriculture, both in terms of cost and green credentials.

Though major advances in molecular and genetic approaches have been made in recent years, creating elite plant lines with more desirable characteristics remains a challenge due to information bottlenecks. An emerging field known as plant phenomics is helping to describe the performance of plants in response to specific environmental conditions. While phenomics is of invaluable importance to plant breeders, throughput capacities must be enhanced if it is to make efficient use of the data gathered from genotyping activities and help improve future wheat yields.

TAKING PHENOMICS FURTHER

Dr Katia Beauchêne is Phenotyping Lead at the Scientific Affairs Department of the ARVALIS Plant Institute (dedicated to applied agricultural research in France), where she is expanding upon existing knowledge of plant varieties in order to help farmers choose the ones most suited to prevailing and future environmental conditions. The work, under the auspices of the 'Development and utilisation of new generation phenotyping tools to analyse genetic determinants of nitrogen fertilisers use efficiency in bread wheat' (PHENOBLE) project, focuses on development and testing of novel high-throughput phenotyping systems to test crops in relevant agronomic scenarios. The central goal is to coordinate effective development and utilisation of next-generation phenotyping tools to analyse genetic determinants of nitrogen fertilisers use efficiency in bread wheat.

Funded by the French National Research Agency (ANR) and the National Authority for Agricultural and Fisheries Products (FranceAgriMer), and partnered with the French National Institute for Agricultural Research (INRA) and French biotechnology firm BIOGEMMA, ARVALIS is aiming to decipher the genetic factors involved in the interactions between plant environment and genotype to successfully characterise their uptake of nitrogen. As a key factor influencing yield, biomass and protein contain accurate descriptions of plant NUE for precise measurements of complex phenotypic traits. Limited by laborious and destructive techniques, conventional phenotyping practises have not been able to achieve these ends as previous trials have been small scale and inefficient, with samples taking several weeks to collect and analyse. To obtain precise readings of wheat's phenotypic traits it has been proposed that remote sensing by a rapid, automated field platform and metabolomic and transcriptomic measurements be integrated into the existing methods of analysis to monitor the development and growth of wheat crops.

A VIEW FROM ABOVE

Combining automated and metabolic phenotyping techniques for the first time, PHENOBLE is unique. Several projects supported by ANR and INRA are expected to benefit directly from the project's findings on NUE and phenotyping methods at large, which could remove a major obstacle to increasing yields. In the PHENOME project, led by INRA's Dr Francois Tardieu, the development of new field platforms will take their cue from PHENOBLE's automated monitoring system to maximise optical observations of phenotypic traits.

The tractor-borne 'Arche' system relies heavily on advances in remote-sensing technologies over the last decade. Measuring the corresponding green fraction and reflectance of wheat crops in the field from nadir (zenith position) and 57° positions,

four identical spectrometers are fitted onto a boom and measure hyperspectral reflectance. While one spectrometer measures irradiance, another is angled toward the nadir at the zenith position and two sit on either sides of the sampling area. In addition, there are also cameras for measuring the green fraction of leaves. All of Arche's onboard devices are automatically triggered using real-time, centimetre accurate, geolocation technology.

The system has been evaluated in early trials using six cultivars, two seeding densities and three nitrogen levels (low, medium and high) to determine how effectively it can describe the dynamics of leaf area, green leaf area, chlorophyll content and canopy structure in reference to their effects on vegetation indices (VIs – specific combinations of readings from spectral bands strongly related to particular canopy characteristics). The VIs chosen by the team focus on canopy structure while aiming to reduce the effects of variable natural illumination.

The study found that, as expected, VIs were more sensitive to canopy structure when viewed from the nadir position, while the green fraction was a stronger factor at the zenith position. With a large selection of 220 cultivars, two replicates and two nitrogen levels, the second part of the project is aiming to collect these measurements dynamically throughout crop growth for association studies. This study also indicates the Arche system's potential but calls for improvements to its abilities. Having demonstrated the benefits of monitoring the crop canopy, extension of this novel phenotyping method requires field trials in mode variable conditions of abiotic stresses more akin to the diversity of farming environments.

INTEGRATING MEASURES

PHENOBLE has also employed leaf and root sampling to acquire data for metabolomic and transcriptomic analyses in order to describe wheat varieties. In combination, these approaches can be useful tools for describing the interactions between an organism and its environment. With a unique metabolic fingerprint for every cell tissue, they can be used to assess the health of a plant at the molecular level in response to external pressures. ARVALIS has collected 2,700 metabolome and transcriptome samples for analysing the leaves' and roots' gene expression and metabolite profile.

By studying the metabolome and transcriptome destructive samples for molecular markers of NUE in relation to Arche's hyperspectral images, ARVALIS believes that PHENOBLE's ultimate objectives will be achieved when the project concludes later this year – linking the genes responsible for phenotypic changes to the high-throughput, non-invasive remote sensing approaches.

The knowledge acquired through these novel phenomic approaches should lead to improvements in the transfer of nitrogen from the soil into usable forms for the plant (nitrate or ammonia) as well as the ability to better tolerate temporary reductions in nitrogen availability. Furthermore, in conjunction with PHENOBLE, ANR's BREEDWHEAT project also aims to grasp the molecular basis of NUE in its efforts to help French agriculture produce crops that are more tolerant of stressors like nitrogen depletion, a sign of soil infertility.

With PHENOBLE's innovations leading to further developments in BREEDWHEAT and PHENOME, INRA and ARVALIS have a substantial platform from which to disseminate the project's results to the wider scientific community whereby these can be used by wheat breeders to create new, better adapted varieties. Most desirable of all are the benefits that will be conferred on the national and international markets as a result, as Beauchêne summarises: "This will help ARVALIS to better understand variety behaviour and offer better advice to farmers choosing adapted varieties".

The throughput capacities of plant phenomics must be enhanced if it is to improve future wheat yields

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INTELLIGENCE

PHENOBLE

DEVELOPMENT AND UTILISATION OF NEW GENERATION PHENOTYPING TOOLS TO ANALYSE GENETIC DETERMINANTS OF NITROGEN FERTILISERS USE EFFICIENCY IN BREAD WHEAT

OBJECTIVES

- To validate, adapt and improve new phenotyping tools by evaluating a collection of elite wheat lines under field conditions differing in terms of nitrogen regimes
- To provide tools and technologies to: characterise plant performance (including the development dynamics of plant structures and functions); and design high-throughput evaluations of plants in response to desired environmental scenarios, (including novel field techniques based on close range remote sensing)
- To build the first high-throughput phenotyping platform adapted to fine and accurate phenotyping with a new set of tools
- To validate phenotyping tools and establish a set of heritable traits and associated molecular markers

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