



SPICY PEPPERS

Professor Fred van Eeuwijk and SPICY aim at more efficient breeding strategies for complex traits by developing models that produce reliable and robust predictions from solely DNA profiles and environmental characterisations

SPICY (SMART TOOLS for Prediction and Improvement of Crop Yield) is an EU funded research project coordinated by Wageningen University (WU), Netherlands, under the leadership of Professor Fred van Eeuwijk, in collaboration with VIB-Gent, Belgium, INRA-Avignon, France, BME-Budapest, Hungary, PRI-Wageningen, Netherlands, BioSS-Edinburgh, UK and EEFC-Almeria, Spain. The initiative was established in 2008 and aims to create a generally applicable protocol for the prediction of crop yield and quality from DNA profiles and environmental characterisations. Traditional crop growth models aiming at prediction of yield and quality for an hypothetical, average genotype, will be upgraded to facilitate genotype specific predictions. The inputs for these upgraded models consist in, firstly, genotype specific physiological parameters that are stable across changing environmental conditions, and secondly environment specific soil and meteorological characterisations that allow a precise reconstruction of individual genotypic performance for whichever environmental conditions. When DNA polymorphisms can be identified that are associated with physiological parameters, these polymorphisms can be used as input parameters instead of the physiological parameters.

The objectives of SPICY are to identify 1) stable physiological parameters underlying yield and quality (PRI, WU); 2) the genetic basis of the physiological parameters (INRA, PRI, VIB, WU); 3) the critical environmental factors driving yield and quality (PRI, WU); 4) a model formulation that predicts genotype specific performance for a wide range of genotypes and environments, including untested genotypes and environments (PRI, WU). As the desired stable physiological parameters may be hard to measure directly, approximations to the required physiological parameters will be generated from fluorescence measurements taken with an especially designed tool (BME), and from image analysis measurements, again recorded with a SPICY proper tool (BioSS, PRI). To calibrate the crop growth models, experiments are conducted in the Netherlands (PRI) and Spain (EEFC).

The SPICY model predictions should be more precise, more widely applicable, and more cost and resource efficient, thereby contributing to sustainable and competitive agriculture. SPICY's choice of crop is pepper, stemming from the desire to be able to discuss the design and progress of the research with experts at breeding companies, something thought to

be easier for a less competitive crop as pepper than for a highly competitive crop as tomato. Furthermore, high quality genetic, genomic and experimental resources could be made available for pepper.

THE PROBLEM, SOME BACKGROUND, AND AN INTEGRATED APPROACH

To address changing consumer and environmental needs, plant breeders develop new genotypes of crop species with improved genetic properties, e.g. superior yield and quality. The observable characteristics, or traits, constitute the phenotype. Yield is a phenotypic trait that can be observed on a collection of genotypes belonging to a crop species. Differences in yield originate from genetic and environmental differences, but phenotypic differences between genotypes under equal environmental conditions should be genetic.

An important modern breeding tool is given by molecular markers. Molecular markers are DNA polymorphisms who can be read off by the application of molecular genetic techniques. For example, in a set of genotypes, half of the genotypes may be scored as 'C' for a polymorphism located at a known position at the chromosomes, whereas the other

CROP GROWTH MODELLING, STATISTICS AND QUANTITATIVE GENETICS

effects. Therefore, the identification of the multiple markers that are associated with a complex trait is far more difficult than the identification of a single marker close to or in the causal gene for a simple trait. Hence the need for more advanced statistical methods to identify such a set of markers. Another major complication is the phenomenon of genotype by environment interaction (GxE): the genetic ranking of genotypes, changes with the environment, i.e., a genotype that is superior under well watered conditions may yield poorly under dry conditions. When GxE occurs, it is not obvious anymore how to do MAS.

SPICY offers a protocol to produce a cost and resource efficient MAS strategy for complex traits. The central idea is to avoid building a MAS strategy for the complex traits that will suffer from strong GxE, and whose genetic base consists of many genes with small effects. Instead the complex trait is dissected in a number of component traits that are stable across environments and that have a simple genetic basis, so that corresponding markers can easily be identified. The critical assumption in the SPICY approach is that it is possible to write variation in a complex trait like yield as a function of a set of environmentally stable component traits and environmental inputs. In addition, the assumption is that the genetic basis of the component traits can easily be identified to produce a set of markers for general use in MAS. To maximise the probability of success, the SPICY team integrates various disciplines and contains experts in each of those disciplines.

Quantitative modeling of phenotypic responses is a central activity in the work of SPICY (WU, PRI). Various crop growth models will be investigated for their possibilities to arrive at environmentally stable parameters. For alternative parameterisations of crop growth models, sensitivity analyses will be performed to quantify the influence of individual parameters on the predictions. For strongly influential parameters, it then needs to be verified whether they are stable and have a simple genetic basis. This identification of the genetic basis is called quantitative trait locus (QTL) mapping. A QTL is a region on a chromosome with a significant contribution to the target trait, DNA polymorphisms within the QTL region will show associations with the trait. A QTL region contains the causal gene and a number of markers that may be used later in MAS. A marker within the causal gene provides the best means for MAS, but standard QTL mapping techniques are not precise enough to distinguish between causal associations and close-marker associations.

SPICY will develop powerful QTL mapping techniques that can map multiple traits simultaneously, taking into account possible correlations between those traits, and GxE. The result of these analyses will be a chromosome map with the positions and effects of QTLs for all traits and an indication whether a particular QTL is stable across environments, Inspection of

half is scored as 'G'. When a particular disease resistance, a phenotypic trait, is found associated with this polymorphism, genotypes showing a 'C' could be more resistant than genotypes showing a 'G'. As a consequence, plant breeders can in future select for this disease resistance by inspecting the status of the DNA for the associated polymorphism, without having to measure the disease phenotype, or without even having to grow the plants. We then have a molecular marker for disease resistance. Selection of superior genotypes by inspection of markers is called marker assisted selection (MAS). A marker can be associated with a trait without having a causal relation with the trait. The marker merely needs to be predictive of the trait.

MAS by single markers works well for simple traits caused by a single gene with a strong effect that is stable across environments. Many phenotypic traits of commercial interest, like yield and quality, are complex; they are dependent on many genes that are sensitive to the environmental conditions. MAS strategies for simple traits do not work for complex traits, as complex traits are caused by many genes with relatively small

CAPTION



SPICY

SMART TOOLS FOR PREDICTION (AND) IMPROVEMENT OF CROP YIELD

OBJECTIVES

- Integrated statistical-genetic & crop-growth models for reliable and robust prediction of complex phenotypic traits, with the example of yield, on the basis of genotype-specific, stable physiological parameters and environmental characterizations
- Molecular markers that are strongly associated with yield and physiological parameters, preferentially located inside causal genes, obtained by a combination of molecular genetics and bio-informatics
- Dedicated high throughput fluorescence and image analysis tools to reconstruct growth and assimilation patterns for individual genotypes over the growing season

PARTNERS

WU, Wageningen University

INRA-Avignon, Institut National de la Recherche Agronomique-Avignon

VIB, Flemish Institute for Biotechnology, Department of Plant Systems Biology, Gent

BioSS, Biomathematics and Statistics Scotland

PRI, Plant Research International/ Praktijkonderzoek Plant & Omgeving (PRI)

BME, Budapest University of Technology and Economics

EEFC, Estación Experimental de la Fundación Cajamar

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that map tells whether QTLs for yield coincide with QTLs for physiological parameters, image and fluorescence analysis results, and gene expressions. The QTL map is an important tool in deciding on the success of replacing MAS for yield by MAS for physiological parameters, or their image and fluorescence analysis proxies. QTLs for gene expressions that coincide with QTLs for yield or physiological parameters are instrumental in finding markers within causal genes.

PHENOTYPING EXPERIMENTS

Quality data is a prerequisite for all modelling. In the second year of SPICY four large-scale phenotyping experiments were performed, in The Netherlands (PRI) and in Spain (EEFC). In these experiments 149 genotypes of a Recombinant Inbred Line (RIL) population were characterised, as well as the parents, the hot pepper CM 334 and bell pepper, Yolo Wonder, and the corresponding F1. INRA-Avignon kindly made this population available to SPICY. Measurements included leaf area and weight of stems, leaves and fruits at the beginning and end of the season, and length, number of fruits on the plant, number of internodes and weights of harvested fruits over the season.

FLUORESCENCE SENSOR

Measurement of the photosynthesis process by a dedicated fluorescence sensor can provide information on the potential assimilation rate of genotypes but current fluorescence measurement techniques are slow. In SPICY, BME developed a fluorescence sensor with multiple measuring heads and a shortened protocol. The sensor has a central computerised unit which controls several intelligent heads, each capable of autonomously carrying out a single fluorescence measurement upon request from the controller and transferring the data to the central unit. Prototypes of the sensor have been tested and in future the sensor should be capable of steering five to ten measuring heads with wireless data collection, thereby further reducing the time necessary to characterise large numbers of plants.

IMAGE ANALYSIS

An image recording platform has been created for automatic large scale recording of the plants while growing in the greenhouse. The aim is to extract features from the images that are related to important plant properties. Such properties are too

time consuming and expensive to measure at present. The imaging platform contains twelve cameras and consists of colour images, infrared images and so-called range images, with the latter correcting perspective errors. The platform has already produced hundreds of gigabytes of data, which now are being analysed and for which new algorithms are developed by BioSS and PRI.

DNA-MARKERS WITH GUARANTEED ASSOCIATION WITH PHENOTYPIC TRAITS

The best markers for MAS are located within the causal genes underlying a trait. To find such markers, INRA-Avignon and VIB will investigate related species of pepper (Solanaceae), for their yield-affecting genes and then try to identify pepper homologues. Within those homologues, DNA polymorphisms point to candidate markers, for yield or physiological traits. Furthermore, gene expression will be studied on the RIL population. The gene expression levels will be mapped as if they were quantitative traits to identify regions in the genome where yield QTL coincide with gene expression QTL. The genes underlying the expression variation may then be identified as 'causal' genes for yield and polymorphisms inside those genes can be used as reliable markers.

THE FUTURE

SPICY aims at prediction of complex traits for a range of genotypes and environments on the basis of stable physiological parameters, or their marker or high throughput proxies, and a set of environmental inputs, thereby reducing the need for phenotyping of new genotypes. SPICY has a duration of four years, two of which have passed. Phenotyping experiments have just been completed and QTL analysis is now in progress. Around 600 potential candidate markers for MAS have been generated and prototypes for image analysis and fluorescence techniques are in use, while initial versions of integrated statistical-genetic and crop growth models are already producing predictions.

Achievements have been discussed with the Industrial Advisory Board at the second annual progress meeting in March. In the remaining years three and four, the SPICY team expects to fully realise its aims and objectives. The multidisciplinary approach that SPICY is creating demonstrates real potential to make a significant impact on European breeding in general and European pepper cultivation in particular.

