European Plant Phenotyping Network

EPPN 2020

D2.3: Statistical methods and software for analysis of single and multiple platform experiments

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This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 731013. This publication reflects only the view of the author, and the European Commission cannot be held responsible for any use which may be made of the information contained therein.

Document information

EU Project N°	731013	**Acronym**	EPPN2020
Full Title	European Plant Phenotyping Network 2020		
Project website	www.eppn2020.plant-phenotyping.eu		

Deliverable	**N°**	D2.3	**Title**	Statistical methods and software for analysis of single and multiple platform experiments
Work Package	**N°**	WP2	**Title**	Design and analysis of phenotyping experiments across multiple platforms, scales of plant organisation, traits and management conditions

Date of delivery	**Contractual**	30/04/2020 (Month 36)	**Actual**	17/06/2020 (Month 38)	
Dissemination level	X	**PU Public, fully open, e.g. web**			
		CO Confidential, restricted under conditions set out in Model Grant Agreement			
		CI Classified, information as referred to in Commission Decision 2001/844/EC.			

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Version log			
Issue Date	**Revision N°**	**Author**	**Change**
29/04/2020	0	Emilie Millet	first version
30/04/2020	1	Fred van Eeuwijk	first review by WP leader
05/05/2020	2	Emilie Millet	second version
08/05/2020	3	Fred van Eeuwijk	second review by WP leader
18/05/2020	4	Cloé Paul-Victor	Reviewed by project manager
15/06/2020	5	François Tardieu	Reviewed by Coordinator
16/06/2020	6	Emilie Millet	Final version

Executive Summary

Objectives: Develop a user-friendly statistical toolbox to analyse single and multiple phenotyping experiments. Provide instructions for the use of particular statistical models and techniques as well as make available suitable and reliable software.

Rationale: We developed a classification of phenotyping data that help define appropriate statistical analyses techniques and software. A pipeline starts with unorganised data as occurring in images and spectra collected by phenotyping devices and ends at predictions of complex traits with genotype by environment interactions.
The statistical methodology is based on linear mixed models and spline technology. A first step in the analysis of time series at plant level is spatial adjustment for individual time points. For this purpose, two-dimensional spline technology is used. The next step consists in fitting spline functions for dynamical trait behaviour at plant level to the spatially adjusted data. From the splines, data summaries describing the dynamics of the fitted curves are extracted as means, slopes, accelerations, minima, maxima, etc. These dynamic parameters are subsequently used as response in mixed models for estimating genotypic effects and heritability. When multiple experiments are performed with a common genotypic panel across different environmental conditions, statistical analysis methods for genotype by environment interaction are suitable for analysing the environment dependence of the dynamic parameters.

Main Results: Statistical methods, software, protocols and teaching material for the analysis of single and multiple phenotyping experiments. The R procedure statgenHTP corrects time series data as obtained at phenotyping platforms for spatial trends and estimates genotype and plant specific dynamics' parameters. Environmental dependence of these dynamical parameters can be investigated by the R procedure statgenGxE. Descriptions of the methods, illustrations of use and software were tested in courses and are available at the intranet site of EPPN2020

Authors/Teams involved: Emilie Millet (WU) & Fred van Eeuwijk (WU)

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1. INTRODUCTION

1.1. Aim of the EPPN2020 project

The EPPN2020 project aims at providing public and private European plant scientists access to a wide range of state-of-the-art plant phenotyping facilities, techniques and methods. It will help the plant community in progressing towards excellence across the whole phenotyping pipeline that includes sensor and imaging techniques, data analysis adjusting treatment contrasts for environmental conditions and placing interpretation in a biological context, data organization and storage, and analysis of series of experiments as well as meta-analyses of experiments.
EPPN2020 coordinates its activities with the future infrastructure EMPHASIS, listed in the ESFRI roadmap, and with national programs. EPPN2020 involves:

- access to 31 key installations in 15 infrastructures,
- a Work Package on sensors (WP1),
- a Work Package on data analysis (WP2),
- a Work Package about data management (WP3),
- networking activities for establishing cooperation and increasing integration between facilities both within and outside EPPN2020.

1.2. Scope and aim of the document

We have introduced a conceptual classification of new phenotyping traits and defined the steps required to integrate them into genotype-to-phenotype (G2P) models (van Eeuwijk et al., 2019). Phenotypic information is classified into five classes, see Figure 1. At the lowest level, level 1, data are collected by phenotyping devices, e.g. images and spectra. The raw phenotyping data need to be converted into interpretable plant features before any statistical analysis can be applied. Machine learning techniques, including deep learning, are used to convert the raw phenotyping information from images and spectra into plant features. Besides, indices can be used to compress the information from spectra into a limited number of features. In JRA2, we concentrate on statistical procedures for analyzing phenotyping data after the stage of feature extraction.

Extracted plant or experimental-unit specific features form the data at level 2 of our data hierarchy, level 2 phenotypes. These features typically are measured (extracted) multiple times over the course of a phenotyping experiment and therefore provide a time series. In this document, we present methodology to correct the level 2 data (extracted features) for spatial heterogeneity and subsequently fit a statistical model to the corrected level 2 phenotypic data as a function of time. From this analysis, curve characteristics are saved that represent level 3 phenotypic data. Level 3 phenotypes can be means, intercepts, slopes, accelerations, inflection points, maxima, minima, asymptotes, and more that describe the time dependence at a plant level of an extracted feature, level 2 phenotype, that was spatially adjusted.

The level 3 phenotypic traits, mainly dynamical parameters of extracted plant features, are further modelled in relation to environmental conditions. These conditions can be qualitative, like stress versus non-stress across multiple experiments in time and space. But, the environmental conditions can also be quantitative and collected with sensors. The estimation of the dependence of dynamical plant traits on environmental conditions produces level 4

phenotypic traits: rates of change of dynamic plant traits in relation to environmental conditions. The presentation of analysis methods for level 3 traits and the estimation of level 4 phenotypic traits is another part of D2.3.

After the estimation of level 4 traits, we can model complex target traits, i.e., traits with an elaborate and complex genetic architecture and environmental dependencies as functions of multiple level 4 traits and environmental inputs. Complex traits represent level 5 traits. Prediction models for level 5 traits can be regression type of models with as inputs multiple level 4 traits and environmental characterizations. Level 5 traits can also be modelled in network types of models with directed edges. Models for level 5 traits form the content of D2.6 and will not be discussed here.

For D2.3, we present here methods and software to model level 2 phenotypes, traits obtained after feature extraction. We propose a two-stage modelling approach. The first stage consists of fitting a spatial model for the level 2 phenotypic data at each time point. The level 2 phenotypic data is "corrected" by subtracting estimates for spatial variation and variation due to statistical design factors (blocks, rows, columns). This strategy allows one to keep the data resolution at the plant or experimental plot level, making analyses simpler. In the second stage, the corrected phenotype is modelled in relation to time, where level 3 phenotypes, i.e., curve parameters and characteristics are estimated. Finally, we present classical multi-environment analysis methods for analysing the curve characteristics across environmental conditions.

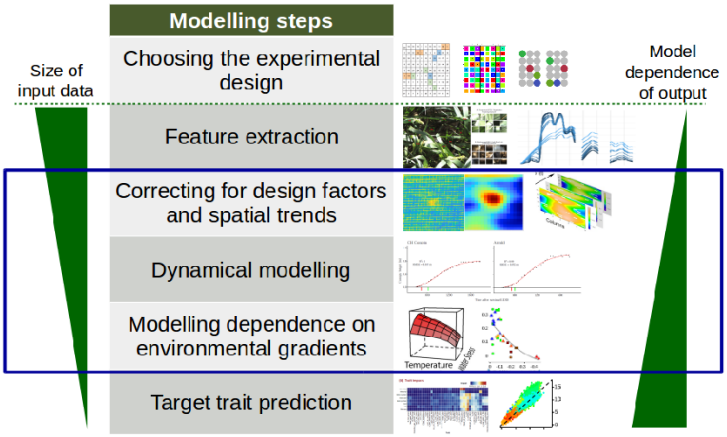

Figure 1. Modelling steps that convert raw phenotyping data, level 1 phenotypes, into model dependent predictions. Dimensionality of input data decrease because data are replaced by integrative parameters that become increasingly model dependent. D2,3 focuses on statistical methodology for the boxed stages. Adapted from van Eeuwijk *et al.* 2019.

2. A TWO-STAGE PROCEDURE FOR THE ANALYSIS OF PLATFORM EXPERIMENTS

The ***statgenHTP*** package was developed as an easy-to-use package for analysing level 2 phenotypic data coming from high throughput phenotyping (HTP) platform experiments. It was developed within the EPPN2020 project to meet the needs for automated analyses of HTP data. It provides many options for (1) checking and visualising the level 2 data and (2) model the spatial trend with two engines (SpATS and ASReml-r). The dynamical modelling is available in the R package *mgcv* (Wood *et al.*, 2017).

This document presents an example of a two-stage analysis with these tools. The example presented here contains data from an experiment on the Phenovator platform (WUR, Netherlands, (Flood *et al.*, 2016) **Fig.2A**) with Arabidopsis plants. It consists of one experiment

with 1440 plants grown in a growth chamber. The number of tested genotypes is 192 with 6-7 plant replications per genotype. Four reference genotypes were also tested with 15 or 30 replications. The studied trait is the photosystem II efficiency ("EffpsII") extracted from images over time (van Rooijen *et al.*, 2017). Two light intensities were applied: low light in the beginning of the experiment, followed by a period of high light and back to low light until the end of the experiment (**Fig.2B**). The dataset called *PhenovatorDat1* is included in the package and is also used in a tutorial sent to the consortium.

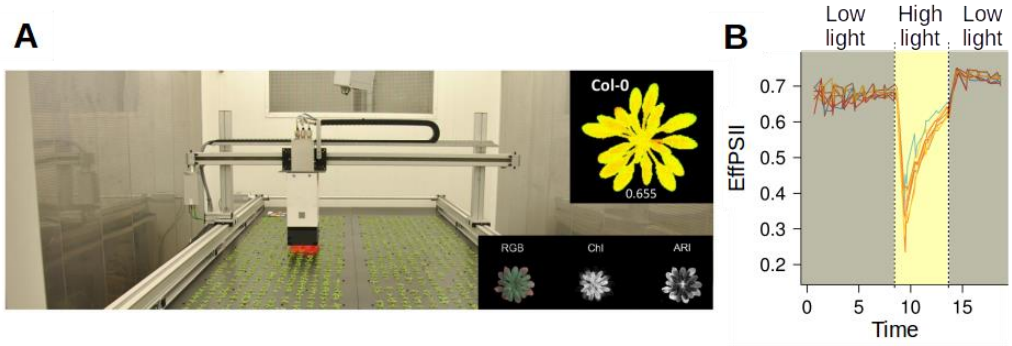

Figure 2. Picture of the Phenovator platform (A) and level 2 phenotype (photosystem II efficiency) for one genotype with multiple plants and an indication of three different light intensity periods (B).

2.1. Data inspection and formatting

The ***statgenHTP*** package provides graphs that help exploring the level 2 phenotypic data. The layout of the experiment can be plotted (**Fig.3A**) to verify the experimental design and define possible statistical models for fitting. Correlations between observations made at pairs of time points can be visualised by a heat plot (**Fig.3B**). Time series data can be visualised for the individual plants for a selection of genotypes (**Fig.3C**). WP2 has also developed a procedure for outlier detection in level 2 phenotypic data (deliverable D2.2, with a tutorial sent to the consortium).

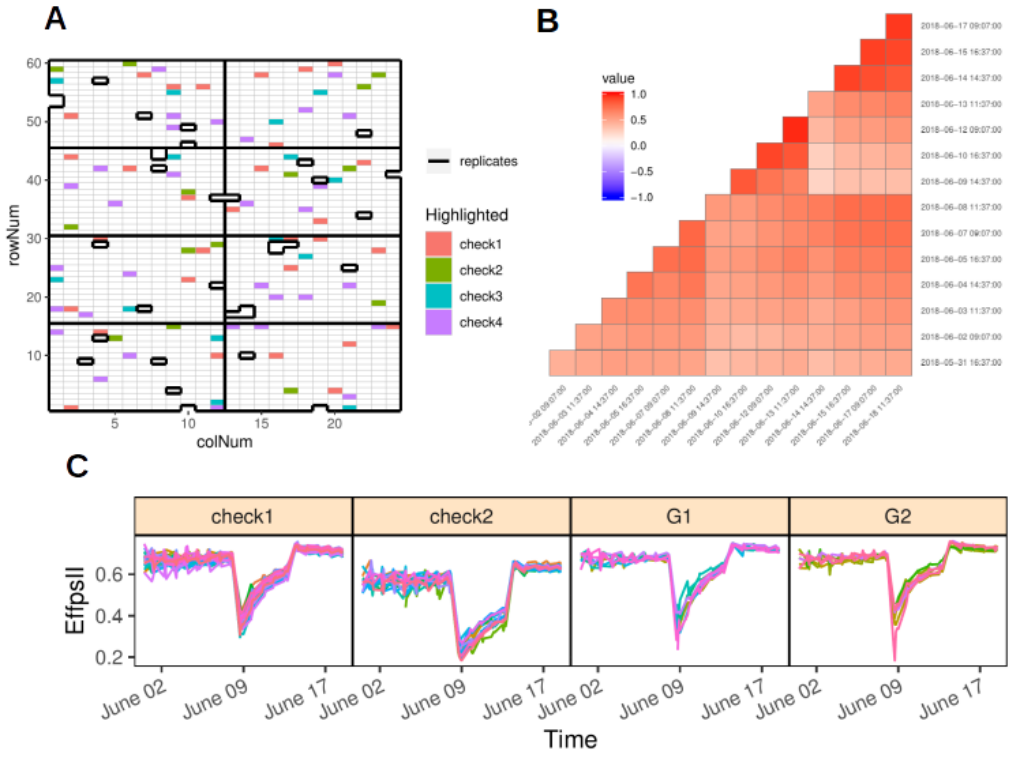

Figure 3. Data exploration using the statgenHTP package. A, plot of the layout of the experiment at one time point with the check genotypes highlighted in colour and the replicates boxed by black lines. B, heatmap of the correlations between time points. C, time courses for plants belonging to a genotype for four genotypes with different colours for the plants.

2.2. Correcting for experimental design factors and spatial variation

Methods to correct for spatial trends in field trials are equally effective for the correction of spatial trends in platform data. In particular, the SpATS model (Rodríguez-Álvarez *et al.*, 2018) was used on many platform data sets (**Fig.4**) provided by project partners and has been proven useful: it can be used in an automatic fashion and it is simple to use (*i.e.* no need for advanced statistical knowledge).

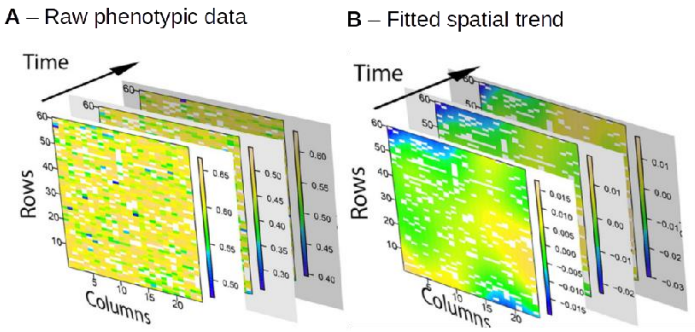

Figure 4. Observed (A) and corrected data (B). Adjustments are made for factors related to experimental design and for spatial variation for each time point using the SpATS model as described in (van Eeuwijk *et al.*, 2019). Data coming from the Phenovator experiment, *PhenovatorDat1*.

After the first checks, the *statgenHTP* program enables modelling for spatial trends using SpATS or ASReml-r (Butler *et al.*, 2017). The *PhenovatorDat1* was modelled using SpATS with row and column effects as well as the spatial term for each time point:

$$y = \mu + Z_g C_g + f_t(u,v) + Z_r C_r + Z_c C_c + \varepsilon \qquad (1)$$

where y is the vector of raw data at one time point, C_g denotes the fixed effect associated with the genotypes, $f_t(\cdot,\cdot)$ is a smooth bivariate surface defined over the row and column positions u and v, C_r and C_c are, respectively, the random effects associated with the rows and columns and ε is the residual ($\varepsilon \sim N(0,\sigma^2)$). Several plots are available to investigate the spatial patterns (**Fig.5 A, B**) and to assess genotypic variability (**Fig.5 C, D**).

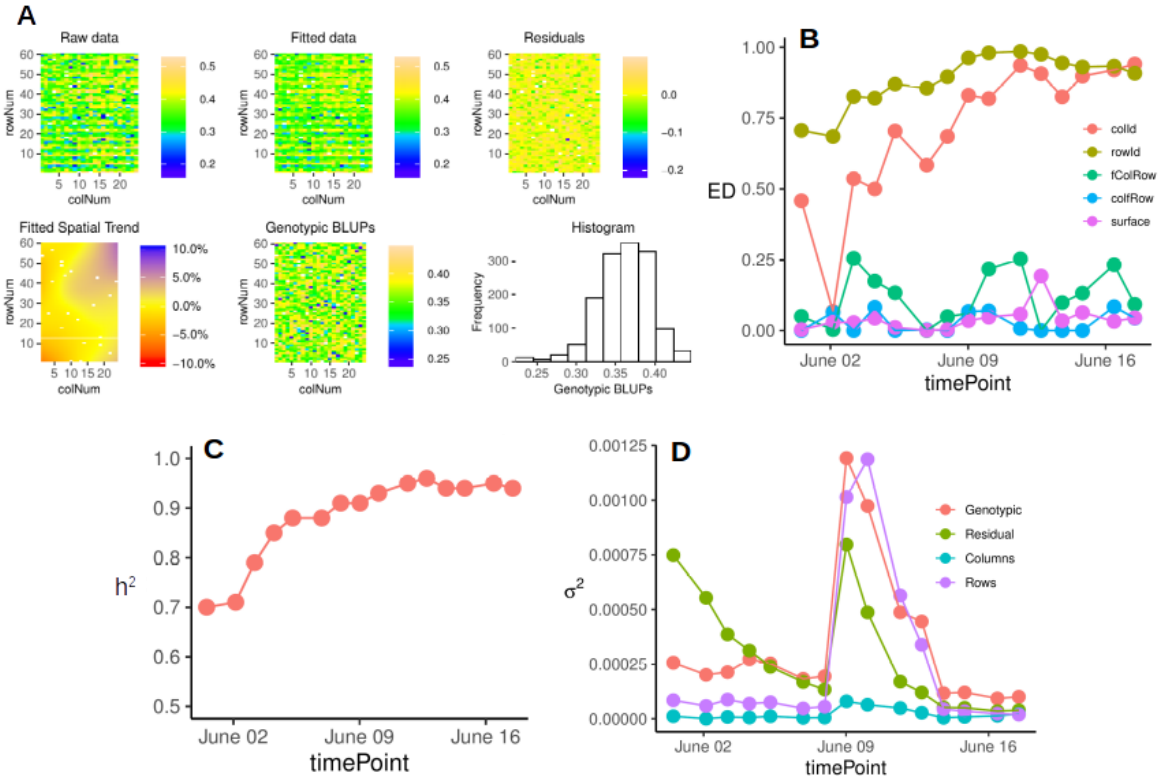

Figure 5. Diagnostic plots available in the statgenHTP R package. A, plot of the spatial trend at one time point. This is inspired by the output of SpATS with heatmaps of the level 2 data, the independent residual and the fitted spatial pattern, together with the genotypic values (here, Best Linear Unbiased Predictors, BLUPs). B, evolution of the effective dimensions of each spatial component over time. C, generalized heritability estimated at each time point. D, variance components estimated at each time point.

In the SpATS approach, we estimate the effective dimensions (ED) of each spatial component at each time point (**Fig.5B**) (see (Rodríguez-Álvarez *et al.*, 2018) for more details). ED can be interpreted as a measure of the complexity of the corresponding spatial component, between 0 and 1, with a value of zero indicating that the component does not contribute to the phenotypic variability. The evolution of the fitted spatial trends over time can also be used as a diagnostic for the quality of the phenotyping installation and this information can help to choose better experimental designs for future phenotyping experiments.

After fitting model (1), the phenotype, y, is corrected by removing the smooth spatial trend and the row and column effects:

$$\tilde{y} = y - \hat{f}_t(u,v) + Z_r \hat{C}_r + Z_c \hat{C}_c \qquad (2)$$

Adjustment of phenotype for experimental design and spatial variation is done per time point. The next step is to model the corrected phenotype (or the genotypic means) in relation to time.

2.3. Dynamic modelling of spatially adjusted secondary phenotypes

To analyse dynamic data, functional analysis is usually carried out by applying a mathematical function able to follow the curve pattern. For well behaving time series, parametric growth models may provide a good description, such as exponential or logistic functions to fit biomass growth. However, many time series and functional phenotyping data sets are difficult to describe by parametric models. The P-splines offer an attractive alternative as flexible smoothing tool (**Fig.6**, Eilers *et al.*, 2015).

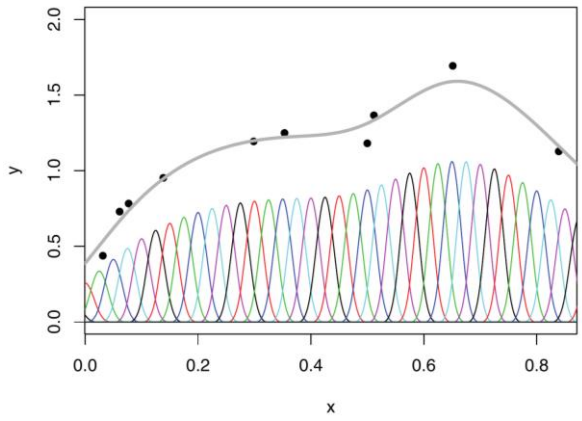

Figure 6. P-spline smoothing of 10 simulated data points with 43 cubic B-spline (from Eilers et al. 2015.

The P-splines were fitted per plant to the corrected level 2 phenotypic data using the function *gam* (generalized additive model) of the *mgcv* package with the REML smoothness selection method (**Fig.7A**). The first derivative was calculated with the *fderiv* package (https://rdrr.io/github/gavinsimpson/tsgam/man/fderiv.html). To summarize the curves, we estimated the following parameters per plant:

- max_slope, which is the speed of recovery of the PSII, calculated from the first derivative of the B-spline basis, during the high light period (**Fig.7B,D**).
- min_EffPSII, which is the minimum photosystem II efficiency, calculated as the minimum value of the EffPSII fitted during the high light period (**Fig.7C**),

A large genotypic variability was observed for the estimated parameters (**Fig.7C,D**). The extracted dynamical parameters can be analysed by simple linear mixed models to estimate genotypic effects for the various defined dynamical parameters and test treatment differences when required.

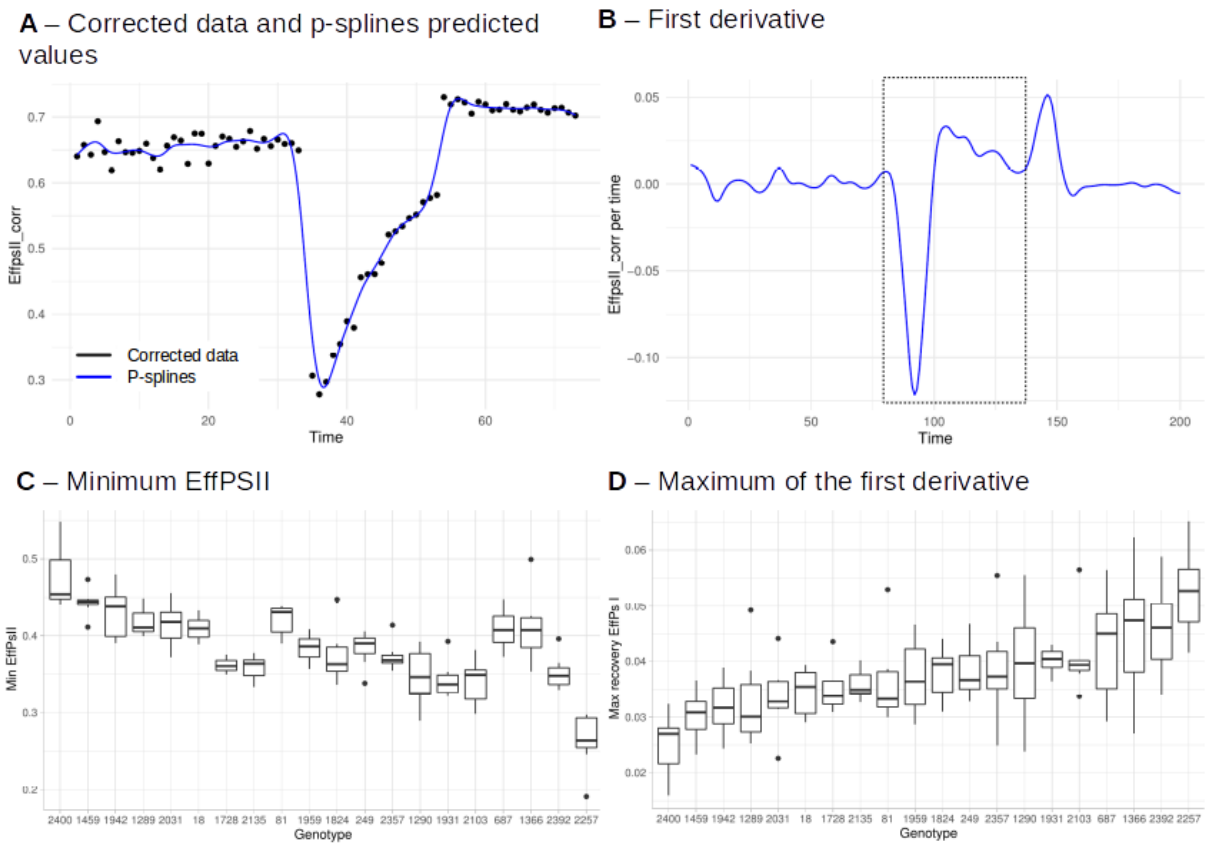

Figure 7. Dynamical modelling of one plant using P-splines on the corrected data (A) and their first derivatives (B). Data per plant are summarized by parameters extracted from the P-spline prediction (minimum values for 20 genotypes, panel C) and the first derivative (maximum values before high light for 20 genotypes, panel D).

3. ANALYSING MULTIPLE PLATFORM EXPERIMENTS

From individual phenotyping experiment analyses, like described in the previous section, we retain genotype specific parameter estimates describing the dynamics of the curves (means, intercepts, slopes, accelerations, minima, maxima, etc.). To analyse the dependence of these dynamic parameters on the environmental conditions, we can perform multi-trial or multi-environment analyses that are common for field data to analyse genotype by environment

interactions (G×E). Therefore, we subject our dynamical parameter to G×E analyses to learn about the environmental sensitivity of our dynamical parameters. For example, we may want to know whether our slopes are stable over experiments done at different times or under increasing levels of an abiotic stress, like drought stress. An overview of methods for G×E analysis is given in van Eeuwijk et al. (2016). These methods are available in the ***statgenGxE*** R package. This package communicates well with the above mentioned ***statgenHTP*** package. The two packages and supporting documents provide a powerful toolbox for analysing individual multiple phenotyping experiments. Teaching material on how to use the packages was tested in an EPPN2020 sponsored course «Statistical tools for plant phenomic data analysis» at the Mediterranean Agronomic Institute Zaragoza (Spain) in January 2020 and is available at the EPPN2020 intranet.

4. FINAL COMMENTS

The statistical analysis of an experiment involving multiple genotypes should separate genetic and treatment (e.g. different irrigations) information from noise variation (environmental variability). In this document, we described protocols and software tools developed in WP2 for an efficient analysis of single and multiple platform experiments. Using quality software for data analysis ensures traceability of decisions.

Beyond the analysis of single and multiple experiments, we worked on a semantic model for the statistical analysis of datasets by linear mixed models (Ćwiek-Kupczyńska *et al*., 2020). Such a sematic model facilitates annotation of datasets and improves their interpretation and interoperability. Using the Statistics Ontology (STATO) to produce FAIR data summaries also enhances experimental data publication and management.

➔ **The tools and methods described in this document have been used in various training courses, including one entitled «Statistical tools for plant phenomic data analysis» at the Mediterranean Agronomic Institute Zaragoza (Spain), in January 2020. We uploaded the teaching material built for this course on the project intranet to make it available to the EPPN2020 consortium. It contains lectures and computer exercises.**

Acknowledgement: This document was written by Emilie Millet (WU) and Fred van Eeuwijk (WP2 leader, WU), with contributions by Bart-Jan van Rossum (WR).

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Glossary

ED: Effective Dimension

EPPN2020: European Plant Phenotyping Network - 2020

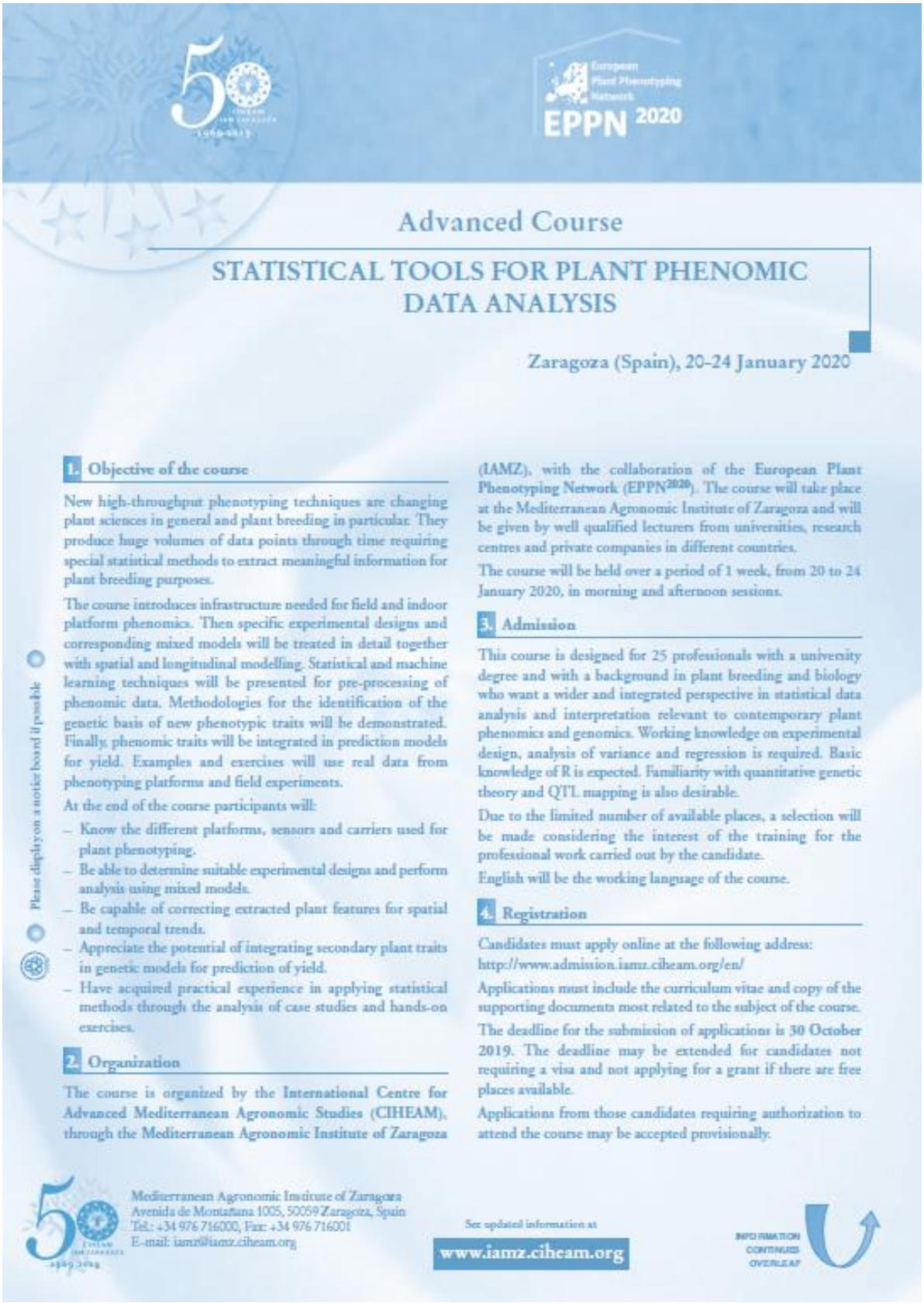
G×E : genotype by environment interactions

HTP: High Throughput Phenotyping

SpATS: Spatial Analysis of field Trials with Splines

STATO: Statistical Ontology

Annex 1: leaflet of the course «Statistical tools for plant phenomic data analysis»



Advanced Course

STATISTICAL TOOLS FOR PLANT PHENOMIC DATA ANALYSIS

Zaragoza (Spain), 20-24 January 2020

Please display on a notice board if possible

1. Objective of the course

New high-throughput phenotyping techniques are changing plant sciences in general and plant breeding in particular. They produce huge volumes of data points through time requiring special statistical methods to extract meaningful information for plant breeding purposes.

The course introduces infrastructure needed for field and indoor platform phenomics. Then specific experimental designs and corresponding mixed models will be treated in detail together with spatial and longitudinal modelling. Statistical and machine learning techniques will be presented for pre-processing of phenomic data. Methodologies for the identification of the genetic basis of new phenotypic traits will be demonstrated. Finally, phenomic traits will be integrated in prediction models for yield. Examples and exercises will use real data from phenotyping platforms and field experiments.

At the end of the course participants will:

- Know the different platforms, sensors and carriers used for plant phenotyping.
- Be able to determine suitable experimental designs and perform analysis using mixed models.
- Be capable of correcting extracted plant features for spatial and temporal trends.
- Appreciate the potential of integrating secondary plant traits in genetic models for prediction of yield.
- Have acquired practical experience in applying statistical methods through the analysis of case studies and hands-on exercises.

2. Organization

The course is organized by the International Centre for Advanced Mediterranean Agronomic Studies (CIHEAM), through the Mediterranean Agronomic Institute of Zaragoza (IAMZ), with the collaboration of the European Plant Phenotyping Network (EPPN2020). The course will take place at the Mediterranean Agronomic Institute of Zaragoza and will be given by well qualified lecturers from universities, research centres and private companies in different countries.

The course will be held over a period of 1 week, from 20 to 24 January 2020, in morning and afternoon sessions.

3. Admission

This course is designed for 25 professionals with a university degree and with a background in plant breeding and biology who want a wider and integrated perspective in statistical data analysis and interpretation relevant to contemporary plant phenomics and genomics. Working knowledge on experimental design, analysis of variance and regression is required. Basic knowledge of R is expected. Familiarity with quantitative genetic theory and QTL mapping is also desirable.

Due to the limited number of available places, a selection will be made considering the interest of the training for the professional work carried out by the candidate.

English will be the working language of the course.

4. Registration

Candidates must apply online at the following address: http://www.admission.iamz.ciheam.org/en/

Applications must include the curriculum vitae and copy of the supporting documents most related to the subject of the course.

The deadline for the submission of applications is 30 October 2019. The deadline may be extended for candidates not requiring a visa and not applying for a grant if there are free places available.

Applications from those candidates requiring authorization to attend the course may be accepted provisionally.

Mediterranean Agronomic Institute of Zaragoza
Avenida de Montañana 1005, 50059 Zaragoza, Spain
Tel.: +34 976 716000, Fax: +34 976 716001
E-mail: iamz@iamz.ciheam.org

See updated information at
www.iamz.ciheam.org

INFORMATION CONTINUES OVERLEAF

Registration fees for the course amount to 500 euro. This sum covers tuition fees only.

5. Scholarships

Candidates from CIHEAM member countries (Albania, Algeria, Egypt, France, Greece, Italy, Lebanon, Malta, Morocco, Portugal, Spain, Tunisia and Turkey) may apply for scholarships covering registration fees and full board accommodation.

Candidates from other countries who require financial support should apply directly to other national or international institutions.

6. Insurance

It is compulsory for participants to have medical insurance valid for Spain. Proof of insurance cover must be given at the beginning of the course. Those who so wish may participate in a collective insurance policy taken out by the IAMZ, upon payment of the stipulated sum.

7. Teaching organization

The course requires personal work and interaction among participants and with lecturers. The international characteristics of the course favour the exchange of experiences and points of view.

The course will be taught through a combination of lectures, case studies and supervised computer practicals.

8. Programme

1. **Prediction, Prescription, Precision and Plant Phenotyping** (1 hour lecture)
2. **Introduction to phenomics** (2 hours lectures)
   - 2.1. Indoor platforms
   - 2.2. Field phenomics including sensors and carriers
   - 2.3. Data processing pipeline for crop phenomics
   - 2.4. The European Plant Phenotyping Network and EMPHASIS
3. **Choosing the design for field and platform experiments** (3 hours lectures + 1 hour practicals)
   - 3.1. Fully replicated experiments
   - 3.2. Partially replicated experiments
   - 3.3. Design of multi-environment trials
   - 3.4. Practical work
4. **Data collection and handling** (1 hour lecture + 2 hours practicals)
   - 4.1. Introduction to feature extraction
   - 4.2. Case study and practical work on date-time formats and relational data tables
5. **Mixed models analysis of extracted features** (4 hours lectures + 4 hours practicals)
   - 5.1. Single experiment
   - 5.2. Multiple experiment
   - 5.3. Analysis of time series
   - 5.4. Case studies and practical work
     - 5.4.1. Correcting for spatial variation and temporal modelling
     - 5.4.2. Rerandomization in platforms
6. **Statistical and machine learning techniques for feature extraction** (4 hours lectures + 2 hours practicals)
   - 6.1. Dimension reduction and cluster analysis
   - 6.2. Penalized regression, classification and regression trees
   - 6.3. Deep learning
   - 6.4. Case study and practical work on feature extraction from hyperspectral canopy reflectance data using indices and multivariate analyses
7. **Environmental data for modelling phenomic data** (2 hour lectures + 2 hours practicals)
   - 7.1. Recording of environmental variates for plant phenotyping experiments
   - 7.2. Construction of environmental indexes
   - 7.3. Case study and practical work on modelling growth and developmental processes using environmental covariates
8. **Integration of environmental, genomic and phenomic data** (4 hours lectures + 4 hours practicals)
   - 8.1. QTL and association mapping
   - 8.2. Genomic prediction
   - 8.3. Use of crop growth modelling

GUEST LECTURERS

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