University of West Hungary
Faculty of Forestry
Pál Kitaibel Doctoral School of Environmental Sciences
Bioenvironmental Sciences

Theses of doctoral (PhD) dissertation

CORRELATION MONITORING TO CHARACTERIZE THE INFLUENCE OF ENVIRONMENTAL FACTORS ON PLANTS

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The choice of subject and the aims of the dissertation

To quantify the effects of the altering environmental factors on plants gets more and more important because of climate changing. It is necessary to disclose the interaction between plants and environment in order to track the response to the plant stress as well as to investigate plant sensitivity and adaptation mechanisms. The biochemical processes of plants are sensitive and they largely depend on the environmental condition.

The concentrations of the plant metabolites can reflect the actual environmental conditions of the sampling. The linear correlations of biochemical variables in the plant tissues can also show environmental sensitivity. The regression straight line of the correlation relation is being modified in the values of slope, intercept, and coefficient of determination and location of the centre point by the alterations in environmental condition. The alterations in the physiological states of plants are traceable with the monitoring of the linear regressions. This research organically fits to the subject of the HB-LKK system model, which approach the alterations of the states in the environmental condition dependent foliage. The objective of my thesis is to detect and to quantify the effects of the environmental factors on plants.

Pairs of linearly correlating biochemical variables, such as, polyphenol-oxidase – peroxidase enzyme activities, the amount of total phenols – antioxidant capacity, concentrations of glucose – fructose and chlorophyll-a – chlorophyll-b and so on, the chosen linearly correlating concentrations are glucose and fructose. Based on environmental sensitivity of the central carbohydrate metabolism, the alterations of the environmental factors dependent physiological states have been monitored by measuring these two sugar amounts in tree leaves. The monitoring of the state dependent regressions of glucose and fructose concentration pairs has been enable a development of quantification for the interaction between plants and the environment.

Materials and methods

The interaction between plants and their environment has been monitored in the vegetation periods of the years 2010, 2011 and 2012. The sampling has repeatedly been carried out per 7-10 days. To determine the concentrations of the carbohydrates occasionally and in every case of sampling plants, 7–7 leaves have randomly been cut off from the foliage.

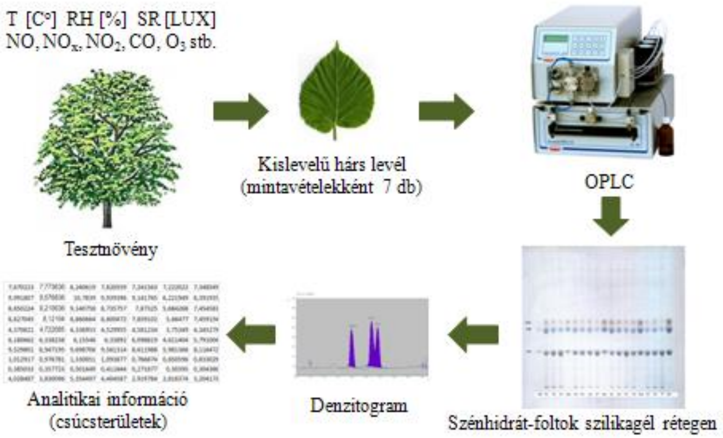

Figure.1. Acquiring the chemical information

At the sampling moment, the values of light intensity illuminating the leaf surfaces have been measured by a sunlight actinometer. To optimize the sampling preparation, previous tests have been executed. According to the optimal preparation method, within 30-40 minutes after the sampling, extracts have been prepared from the individual leaves to separate the carbohydrate contents. The OPLC separation of the carbohydrate compositions (Sárdi et. all, 1996) have been conducted without the storage of the extracts, directly after the centrifuging (figure 1). The chosen trees to the interaction monitoring between plant and its environment were an oak (*Quercus robur*), a beech (*Fagus silvatica* L.) and two linden (*Tilia tomentosa* and *Tilia cordata*) species. The glucose and fructose results of the leaves of the test trees have been associated with the data of the

Sárdi É., Velich I., Hevesi, M., Klement Z. (1996): The role of endogenous carbohydrates in the Phaseolus-Pseudomonas host-pathogene interaction. 1. Bean ontogenesis and endogenous carbohydrate components. Int. Hort. Sci. Hung. 28: 65-69.

meteorology and air pollution in the sampling moment. These data obtained by this way have been constituted the prime database (impute matrix) of the assessment of the interaction between plants and environment.

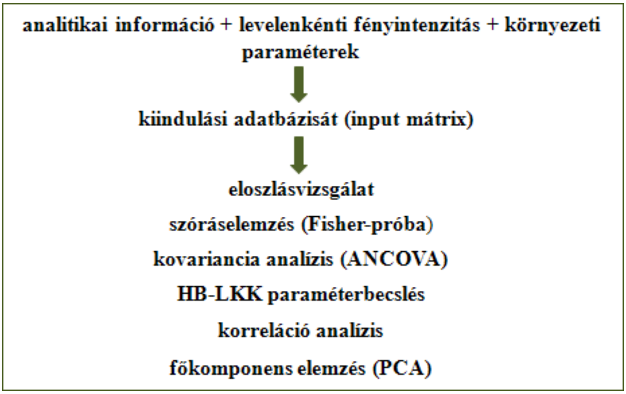

Figure.2. Strategy of assessment

The tests of distributions and standard deviations, covariance (ANCOVA) and correlation analyses and principal component analysis have been applied on the database so as to reveal the latent associations between the measuring outcomes and the environmental factors.

Results of dissertation - Theses

In the most cases, the relative large values of the standard deviations originating from the distributions of the biochemical variables do not allow the significant detection of the effect of environmental factors on foliage by using univariate statistical methods. The methods of the multivariate data analysis more and more come to the front to detect the information hidden in the environmental data. The background knowledge of physics, biology and chemistry are necessarily required for the proper interpretation of the results out of the data analysis. The assessment of multivariate aspect of state dependent regressions has been appropriate to disclose the fine structure of interactions between plants and the environment.

THESIS.I. **The means and the standard deviations of glucose and fructose are correlated to each others with high Pearson R values. The elevations of the glucose and fructose concentrations consort with the elevations of their standard deviations. The raise of slope of glucose – fructose regression straight lines always come with a decrease in the intercept.**

The correlation analysis of the glucose and fructose data in the case of four trees and three vegetation periods highlighted to the positive correlation between the standard deviations and the means. If an arbitrary environmental factor is able to increase the expected values of glucose and fructose distributions, then the standard deviations of the distributions will likely increase, too. The higher the expected values, the higher the standard deviations will be likely. The higher the glucose concentration, the higher fructose concentration, too. The experienced negative correlation between the state dependent regressions slope and intercept is accordance with the meaning of the „theoretical equation of plant-environment interaction". The correlation between the slope and the intercept of regression of glucose and fructose concentrations is an experimental confirmation of the theory.

THESIS.II .**The distributions of glucose and fructose concentrations which are integrated in the state dependent glucose-fructose regressions are the indicators of the interaction between plant and the environment.**

The amounts of glucose and fructose in the investigated foliages can approximately be considered a kind of normal distributions. The actual concentrations of glucose and fructose in the individual leaves are synchronously regulated by the centre carbohydrate metabolism. The equivalence of the distributions and the synchronously regulated concentrations give an explanation to the high values of R^2 of the experienced regression straight lines of glucose-fructose correlation. The covariance analysis (ANCOVA) has proven the state dependence of glucose-fructose correlations.
The parameters of the state dependent regression are changing along with the environmental factors and it is affirmed by the correlation analysis as well as by the principal component analysis. On the basis

of this establishment, the glucose-fructose regression straight line of the foliage and the supplied distributions can be regarded the indicators of the plant-environment interactions. The slope and intercept of the state dependent regressions can significantly change during the modifications of the environmental factors. The state-dependent regressions of glucose-fructose concentrations pertaining to the significantly distinguishable environmental conditions can be seen in the figure 3.

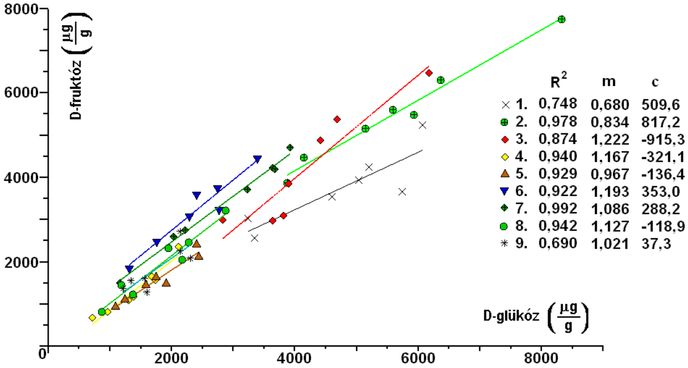

Figure.3. State dependent regressions of the small leaf lime in the vegetation period of the year 2012
(**1.** 24.05.2012; **2.** 20.06.2012; **3.** 05. 07.2012; **4.** 19.07.2012; **5.** 01.08.2012.; **6.** 22. 08.2012; **7.** 27. 08.2012; **8.** 06. 09.2012; **9.** 20. 09.2012)

THESIS.III. **Transformation *f*(.) of HB-LKK system modelling the plant foliage is necessarily linear transformation operator.**

The process of the photosynthesis meets the law of material conservation, thus which one must be valid for the HB-LKK model of the foliage, too. In the photosynthesis, the transformation of the photons to the electrons in chemical bond cannot deviate from the linear one because it would go against the law of the material conversation.

Applied for the description of biological systems, the transformation function $f(.)$ of equation (1) in the HB-LKK model must be linear operator.

(1) $$y_1(t) = a_1 f(u(t)) + b_1 \qquad y_2(t) = a_2 f(u(t)) + b_2$$

In this way the system functions of HB-LKK can be reduced to the form equation (2).

(2) $$y_1(t) = a_1 \cdot u(t) + b_1 \qquad y_2(t) = a_2 \cdot u(t) + b_2$$

Divided by light intensity, we can obtain the equations (3) from the equation (2).

(3) $$\frac{y_1(t)}{u(t)} = a_1 + b_1 \cdot \frac{1}{u(t)} \qquad \frac{y_2(t)}{u(t)} = a_2 + b_2 \cdot \frac{1}{u(t)}$$

The parameters b_i in the HB-LKK system function reflects the effect of the environmental conditions on the variables y_i. The parameters a_i are system parameters that play a role in the formation of the variables y_i. This means how big part of variable u is used to configure the values of y_i. Weighted the amounts of glucose and fructose with light intensities and correlated them to the reciprocal values of light intensity, the values of carbohydrates concentrations per the unit light must produce linear regression in the function of reciprocal light intensity.

The validity of the equation (3) can be checked with regression analysis. For example, applied the HB-LKK system functions to the correlation monitoring data of small leaves lime and fitted straight lines to data pairs of variables glucose/light (fructose/light) and 1/light, the straight lines depicted in figures 4 and 5 can be obtained.

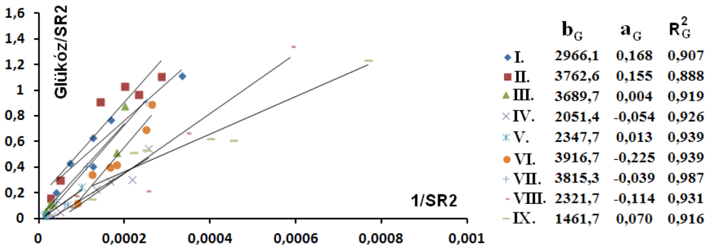

Figure.4. Regression straight lines of variables glucose/light and 1/light intensity

(**1.** 24.05.2012; **2.** 20.06.2012; **3.** 05. 07.2012; **4.** 19.07.2012; **5.** 01.08.2012; **6.** 22. 08.2012; **7.** 27. 08.2012; **8.** 06. 09.2012; **9.** 20.09.2012; Regressions without outliers; Bevington crit. (6, 95 %)=0,811)

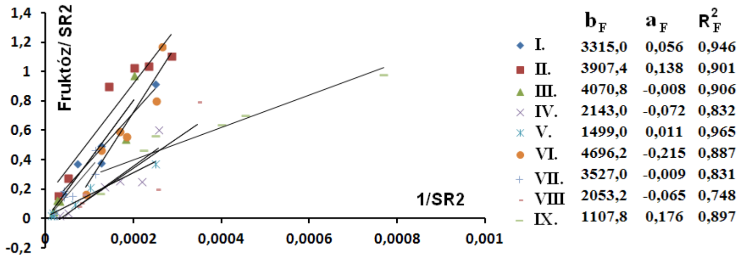

Figure.5. Regression straight lines of variables frucose/light and 1/light intensity

(**1.** 24.05.2012; **2.** 20.06.2012; **3.** 05. 07.2012; **4.** 19.07.2012; **5.** 01.08.2012; **6.** 22. 08.2012; **7.** 27. 08.2012; **8.** 06. 09.2012; **9.** 20.09.2012; Regressions without outliers; Bevington crit. (6, 95 %)=0,811)

The results originated from the central carbohydrate model (Nägele et. all, 2010) and those ones of correlation monitoring investigations support the system function of HB-LKK model to the behaviour of the foliage with significantly linear regressions of y_i/u versus $1/u$. The covariance analysis of these regressions can reveal significantly different regressions modelling the HB-LKK system of different sampling times. The states of the foliage (the changing of states) are characterized by significantly different HB-LKK system parameters.

Nägele T., Henkel S., Hörmiller I., Sauter T., Sawodny O., Ederer M., Heyer A. G. (2010): Mathematical Modeling of the Central Carbohydrate Metabolism in Arabidopsis Reveals a Substantal Regulatory Influence of Vacuolar Invertase on Whole Plant Carbon Metabolism. Plant Physiology 153: 262.

THESIS.IV. **The parameters b_G and b_F of the HB-LKK system model are dependent on the environmental factors.**

The system parameters determined for glucose-fructose values (a_i; b_i) are significantly correlated in linear way to each other. The parameters b_G and b_F have shown some correlation beside high values of *Pearson R coefficient* to the values of temperature, ozone and sulphur dioxide concentrations (I. table). In the theory of "State dependent correlation concept", the parameters b_i of HB-LKK system can model the effects of environmental factors on the resultant values of the variables y_i (concentration of glucose and fructose). The values of parameters b_i and the mentioned environmental conditions alter together. This is the experimental confirmation of the existence of theoretical system functions.

Table.I. The correlation coefficient of Pearson R between the system parameters b_i and the environmental factors

	T	**AH**	**O_3**	**SO_2**	**NO_2**	**NO_x**	**CO**	**PM_{10}**	**NO**	**SR**	**SR2**
b_G	**0,736**	0,528	**0,858**	**0,747**	-0,180	-0,262	-0,189	0,368	-0,365	0,619	0,231
b_F	**0,707**	0,609	**0,874**	**0,763**	0,001	-0,101	-0,018	0,537	-0,240	0,414	0,028

(b_G = transformation parameter of glucose; b_F = transformation parameter of fructose; **T** = temperature [C°]; **AH** = absolute humidity [g/m^3]; **O_3** = ozone [μg/m^3]; **SO_2** = sulphur dioxide [μg/m^3]; **NO_2** = nitrogen dioxide [μg/m^3]; **NO_x** = nitrogen oxide [μg/m^3]; **CO** = carbon monoxide [μg/m^3]; **PM10** falling dust concentration[μg/m^3]; **NO** = nitrogen monoxide [μg/m^3]; **SR** =background solar radiation [LUX];**SR2** = light intensity at individual leaves [LUX])

The HB-LKK system function and the theory of "state dependent correlations" create an obvious relationship between the b_i parameters and the expected values of the distribution. The relationship between the parameters b_i and the means of the carbohydrate concentrations has been confirmed by the regression straight lines with high values of R^2.

THESIS.V. **The coordinate system determined by the first and second principal components can be regard as the interaction space between plant and environment.**

By projecting speckles of the examined leaves into the coordinate system of the first two score components, groups of the leaf objects can be visualized. The score plot of the conjugated data reflects significantly different interaction between the plants and environment. These interactions are independent of both species and vegetation periods (figure 6). The seven and seven leave samples at particular sampling times are located very close to each other. Those sampling times produce specie independent classes that have similar values of environmental factors in the various sampling moments.

Every variables of interaction between plants and environment contribute to the formations of every principal component. From the loading matrix of the principal component analysis, it can be established that the first principal component is mainly shaped by the variables of the plant (carbohydrates) and the second one is rather prevailed by the environmental condition.

In the space of the first two principal components, significantly different state-dependent regressions of glucose and fructose contents pertain to the groups of leaves at different sampling times.

The explained variances of PCA assessment are nearly the same in the cases of both the separated tree data and the set of conjugated data. It strengthens the assumption that the space of the first two principal components involves the potential states of the interaction between plants and environment. The interactions of the plants and environment can be produced in the six dimensions of a subspace with explained variance of about 95% (figure 7). Beside small-scale neglect, the changing of interactions between plants and environment can be modelled with reduced number of principal components.

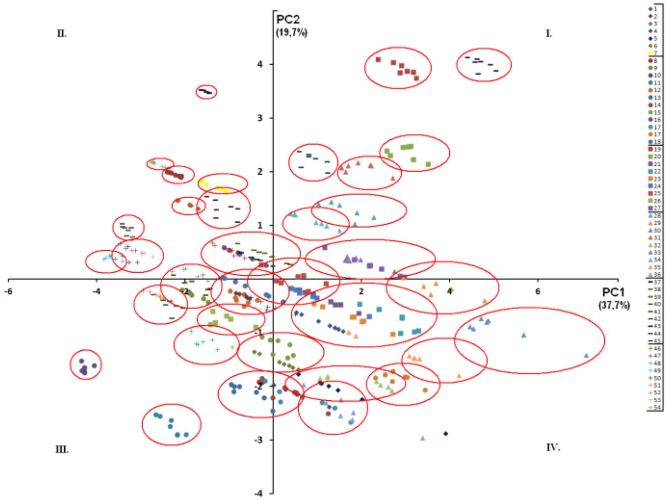

Figure.6. The score plot of the principal component analysis of the conjugated data

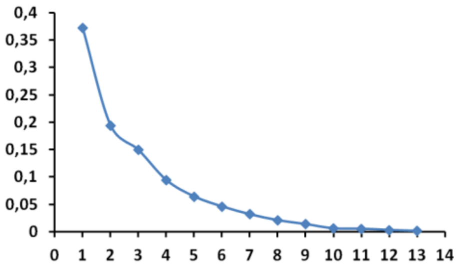

Figure.7. The scree plot of the principal component analysis of the conjugated data

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