# POD-PPO State-dependent Correlation as an Adaptation Indicator in the Vegetation of Forest Trees

Zsolt István NÉMETH – Dorottya Zsófia BADÁCZY

Institute for Chemistry, University of West Hungary, Sopron, Hungary

**Abstract** – The correlation of biochemical variables and the regression of their values are very sensitive to physiological state alterations that make them be suitable for the characterization of the interaction between the plant and its environment and for the indication of plant adaptation ability, respectively. The concept of state-dependent correlation applied for the activities of peroxidase (POD; EC 1.11.1.7) and polyphenol oxidase (PPO; EC 1.10.3.1) was used to monitor the vegetation period of some forest trees. The POD and PPO activities of leaf extracts from Penduncculate oak (*Quercus robur* L.) and beech (*Fagus sylvatica* L.) trees provided linear correlation. Parameters of their state-dependent regressions have been established to be susceptible to the environmental conditions. Comparing the state-dependent regressions belonging to various sampling times to each other, deterministic alterations of POD-PPO regression are revealed that can be related to the alterations of temperature, relative humidity and global solar radiation. Beside the conservation of high values of the coefficient of determination ($R^2$), the vegetation sequence of the state-dependent regressions and the alterations experienced in those ones can be considered a consequence of the adaptation in the plant ecological system.

**Keywords**: state-dependent correlation / peroxidase / polyphenol oxidase / Fagus sylvatica / Quercus robur

## 1. INTRODUCTION

The higher plants have several classes and types of PODs, reaching up to 160 isoenzymes in some plants such as cereals (DUNFORD 1999). The same is true for PPO (MAYER 2006). Peroxidases catalyze the oxidation of organic compounds in the presence of hydrogen peroxide. Polyphenol oxidases (PPO) are the enzymes, which are able to insert oxygen in *ortho* position to an existing hydroxyl group in an aromatic ring, followed by the oxidation of the diphenol to the corresponding quinone. The plant antioxidant enzyme system is proved to be sensitive to environmental effects. The POD and the PPO enzymes have in turn been experienced to be susceptible for some stress effects, too. Correlations among the activities of antioxidant enzymes (GONG et al. 2006, KHOSRAVINEJAD et al. 2008) and between PPO gene expression and stress are also reported (MAYER 2006). Correlations were experienced between the components of antioxidant systems of *Mikania micrantha* (ZHANG et al. 2006), *Brassica chinesis* (YANG et al. 2004), tobacco (GONG et al. 2006) and Penduncculate oak (NÉMETH et al. 2009b, NÉMETH et al. 2009c). A correlation of oxidative activities originated from the leaves of two forest species is shown in this article. Seeking the origin of the correlations, the existential conditions of state-dependent correlation have been declared and a theoretical equation was successfully deduced (see *equation 1*). The linear regressions produced by biochemical variables are the statistical approximations of this theoretical relationship.

$$(1) \qquad x_1 = \frac{\sigma_1}{\sigma_2} x_2 + \frac{\sigma_2\mu_1 - \sigma_1\mu_2}{\sigma_2}$$

$$\mu_i = \lim_{n\to\infty}\left(\overline{x_i}\right) = \lim_{n\to\infty}\left(\frac{1}{n}\sum_{j=1}^{n} x_{i_j}\right), \overline{x_i}$$ is the mean of variable $x_i$,

$$\sigma_i = \lim_{n\to\infty}\left(s_i\right) = \lim_{n\to\infty}\left(\sqrt{\frac{\sum_{j=1}^{n}\left(x_{i_j} - \overline{x_i}\right)^2}{n-1}}\right)$$ and $s_i$ is the $i^{th}$ empirical standard deviation

where, $x_1$ and $x_2$ are the biochemical variables in the correlation (e.g. metabolite concentrations, enzyme activities, etc). $\mu_1$ and $\mu_2$ are the expected values of the variables $x_1$ and $x_2$. $\sigma_1$ and $\sigma_2$ are the theoretical standard deviations of distributions of variables $x_1$ and $x_2$. The linear regressions between some biochemical variables in a given physiological stage are regarded as state-dependent regressions since the dependences of the regression parameters (slope, intercept, $R^2$) from some environmental factors have been experienced and established. The conditions of existence of strong state-dependent regressions are the synchronized regulation of the variables in metabolism and the identity of the types of their distributions. (NÉMETH et al. 2009a). In this work, the concept of state-dependent correlation applied for the activities of peroxidase (POD) and polyphenol oxidase (PPO) were used to track the adaptation of two forest trees within the vegetation period.

## 2. MATERIALS AND METHODS

To tentative study the resultant activities of POD and PPO isoenzymes, two traditional photometrical methods have been chosen (FLURKEY – JEN 1978, SHANNON et al. 1966). *Extraction.* The fresh leaves were powdered with equal quantity of quartz sand. For enzyme extraction, the samples of 1.20 g were extracted with 15.00 ml amounts of K-Na-phosphate buffer (pH = 6.0) solutions at 4 $^{o}$C for 20 minutes and then centrifuged at 6000 rpm for 10 minutes. *Assay for resultant oxidative activity of PPO isoenzymes.* By monitoring of the increase in absorbance at every $10^{th}$ second with a Hitachi U-1500 spectrophotometer at 420 nm (3min), resultant activity of PPO isoenzymes (EC 1.10.3.1) with catechol substrate was measured. Sample: 1 ml buffer (pH=6.0) + 1 ml (0.2 M catechol) + 0.5 ml (extract), (FLURKEY and JEN 1978). 1 PPO Unit = 0.001 absorbance unit·min$^{-1}$. *Assay for resultant oxidative activity of POD isoenzymes.* Resultant activity of POD isoenzymes (EC. 1.11.1.7) with *o*-dianizidine as chromogen reagent was determined by spectrophotometry at 480 nm (4 min). Sample: 1.7 ml buffer (pH=6) + 30 µl (0.3 % $H_2O_2$) + 20 µl ( *o*-dianizidine) + 10 or 20 µl (extracts), (SHANNON et al. 1966). 1 POD Unit = 0.01 absorbance unit·min$^{-1}$. *Protein determination*. The determination of protein amounts in the extracts was carried out with Bradford's method (BRADFORD 1976). *Statistical analysis* The StatsDirect (v.2.7.8) statistical software (www.statsdirect.com) and Scilab (v. 4.1.2) program (www.scilab.org) were applied to evaluate the primary results.

## 3. RESULTS

To investigate the vegetation period of 2007$^{th}$ year, a Pendunculate oak and a beech have been chosen as model trees. The samplings of two trees were at the same times. Five and five leaf samples were gathered from both trees. From May until September, eight samplings were performed. The primary results of the measurements can be found in *Table 1*. The actual meteorological parameters of environmental condition are summarized in *Table 2* together with the means and empirical standard deviations of measured enzyme activities.

*Table 1. POD and PPO activities (U/μg protein) in the vegetation period and their statistic parameters* (SD – standard deviation; CI - confidence interval; $R^2$ – coefficient of determination; The letter of sampling in bold (e.g. (**A**)) indicate equivalent results of Shapiro-Wilk W test of POD and PPO data by StatsDirect software)

| Sampling | **Beech** | 1 | 2 | 3 | 4 | 5 | Mean | SD | ± CI | Slope | Intercept | $R^2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 15 May | PPO | 6.144 | 6.493 | 5.029 | 2.034 | 2.382 | 4.416 | 2.091 | 2.618 | 0.395 | 1.586 | 0.4720 |
| (**A**) | POD | 7.995 | 7.720 | 12.543 | 3.389 | 4.203 | 7.170 | 3.639 | 4.556 | | | |
| 30 May | PPO | 3.064 | 2.574 | 1.963 | 4.173 | 1.922 | 2.739 | 0.930 | 1.164 | 0.340 | 0.617 | 0.9684 |
| (**B**) | POD | 6.554 | 6.482 | 3.974 | 10.437 | 3.776 | 6.245 | 2.692 | 3.370 | | | |
| 12 June | PPO | 0.656 | 0.757 | 0.933 | 1.125 | 0.905 | 0.875 | 0.179 | 0.224 | 0.887 | 0.116 | 0.9573 |
| (**C**) | POD | 0.585 | 0.771 | 0.965 | 1.109 | 0.853 | 0.857 | 0.198 | 0.248 | | | |
| 26 June | PPO | 2.762 | 3.147 | 1.966 | 4.378 | 3.258 | 3.102 | 0.875 | 1.095 | 0.396 | 0.236 | 0.9434 |
| (**D**) | POD | 6.993 | 6.932 | 4.668 | 10.646 | 6.979 | 7.243 | 2.147 | 2.689 | | | |
| 17 July | PPO | 1.840 | 1.714 | 1.777 | 1.983 | 1.706 | 1.804 | 0.114 | 0.143 | 0.336 | 0.508 | 0.9351 |
| (**E**) | POD | 3.988 | 3.483 | 3.875 | 4.332 | 3.639 | 3.864 | 0.328 | 0.411 | | | |
| 31 July | PPO | 5.459 | 3.405 | 1.178 | 3.122 | 1.775 | 2.988 | 1.662 | 2.081 | 0.283 | 0.489 | 0.9939 |
| (**F**) | POD | 17.811 | 10.132 | 2.404 | 8.685 | 5.144 | 8.835 | 5.858 | 7.335 | | | |
| 14 Aug | PPO | 2.994 | 3.134 | 2.153 | 2.795 | 3.059 | 2.827 | 0.397 | 0.497 | 0.281 | 0.591 | 0.9391 |
| (**G**) | POD | 8.593 | 9.357 | 5.699 | 7.987 | 8.191 | 7.966 | 1.371 | 1.717 | | | |
| 28 Aug | PPO | 1.469 | 1.113 | 1.689 | 1.676 | 1.601 | 1.510 | 0.239 | 0.299 | 0.223 | 0.539 | 0.9628 |
| (H) | POD | 4.507 | 2.52 | 4.929 | 5.104 | 4.718 | 4.355 | 1.050 | 1.315 | | | |
| | **Oak** | 1 | 2 | 3 | 4 | 5 | Mean | SD | ± CI | | | |
| 15 May | PPO | 2.538 | 2.045 | 2.105 | 3.300 | 7.028 | 3.403 | 2.088 | 2.614 | 0.376 | -0.632 | 0.9854 |
| (A) | POD | 9.507 | 6.754 | 6.656 | 10.635 | 20.082 | 10.727 | 5.508 | 6.898 | | | |
| 30 May | PPO | 2.099 | 2.568 | 3.179 | 3.045 | 5.943 | 3.367 | 1.502 | 1.881 | 0.285 | 0.915 | 0.9709 |
| (B) | POD | 4.476 | 5.357 | 9.333 | 6.537 | 17.287 | 8.598 | 5.191 | 6.500 | | | |
| 12 June | PPO | 1.437 | 1.577 | 2.228 | 1.430 | 4.829 | 2.300 | 1.451 | 1.817 | 0.315 | 0.408 | 0.9972 |
| (**C**) | POD | 3.489 | 3.970 | 5.546 | 2.985 | 14.068 | 6.011 | 4.605 | 5.766 | | | |
| 26 June | PPO | 5.253 | 5.657 | 7.474 | 7.237 | 8.677 | 6.86 | 1.401 | 1.754 | 0.295 | 2.051 | 0.9946 |
| (**D**) | POD | 10.656 | 12.212 | 18.389 | 18.129 | 22.075 | 16.292 | 4.733 | 5.927 | | | |
| 17 July | PPO | 2.205 | 3.552 | 2.133 | 2.993 | 2.517 | 2.680 | 0.593 | 0.743 | 0.395 | 0.156 | 0.9812 |
| (**E**) | POD | 5.096 | 8.714 | 5.166 | 6.866 | 6.130 | 6.394 | 1.489 | 1.865 | | | |
| 31 July | PPO | 8.710 | 9.026 | 4.696 | 8.692 | 6.018 | 7.428 | 1.952 | 2.445 | 0.348 | 0.117 | 0.8979 |
| (**F**) | POD | 24.156 | 27.228 | 13.455 | 21.857 | 18.399 | 21.019 | 5.319 | 6.660 | | | |
| 14 Aug | PPO | 2.890 | 8.895 | 9.311 | 8.574 | 4.007 | 6.735 | 3.038 | 3.804 | 0.302 | 0.070 | 0.9844 |
| (G) | POD | 10.062 | 29.013 | 29.046 | 29.816 | 12.261 | 22.040 | 9.966 | 12.479 | | | |
| 28 Aug | PPO | 4.842 | 1.460 | 2.056 | 2.673 | 11.707 | 4.547 | 4.201 | 5.261 | 0.288 | -0.657 | 0.9362 |
| (H) | POD | 25.042 | 4.875 | 9.055 | 11.948 | 39.394 | 18.063 | 14.108 | 17.666 | | | |

*Table 2. Means and standard deviations at different environmental conditions* (T – temperature (°C); P – air pressure (kPa); RH – relative humidity (%); GSR – global solar radiation ($MJm^{-2}day^{-1}$).

| | Beech (*F. sylvatica*) | | | | Penduculate oak (*Q. robur*) | | | | Environmental condition | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sampling | $M_{PPO}$ | $SD_{PPO}$ | $M_{POD}$ | $SD_{POD}$ | $M_{PPO}$ | $SD_{PPO}$ | $M_{POD}$ | $SD_{POD}$ | T | GSR | RH | P |
| A | 4.416 | 2.091 | 7.170 | 3.639 | 3.403 | 2.088 | 10.727 | 5.508 | 18.0 | 7.96 | 57 | 101.13 |
| B | 2.739 | 0.930 | 6.245 | 2.692 | 3.367 | 1.502 | 8.598 | 5.191 | 14.0 | 12.30 | 55 | 101.10 |
| C | 0.875 | 0.179 | 0.857 | 0.198 | 2.300 | 1.451 | 6.011 | 4.605 | 35.0 | 15.88 | 37 | 100.15 |
| D | 3.102 | 0.875 | 7.243 | 2.147 | 6.860 | 1.401 | 16.292 | 4.733 | 17.8 | 9.74 | 56 | 100.44 |
| E | 1.804 | 0.114 | 3.864 | 0.328 | 2.68 | 0.593 | 6.394 | 1.489 | 23.3 | 27.69 | 59 | 101.50 |
| F | 2.988 | 1.662 | 8.835 | 5.858 | 7.428 | 1.952 | 21.019 | 5.319 | 17.3 | 18.00 | 62 | 101.82 |
| G | 2.827 | 0.397 | 7.966 | 1.371 | 6.735 | 3.031 | 22.04 | 9.966 | 20.2 | 12.30 | 70 | 101.41 |
| H | 1.510 | 0.239 | 4.355 | 1.050 | 4.547 | 4.201 | 18.063 | 14.108 | 18.1 | 17.53 | 69 | 101.69 |

To study the relations between enzyme activities and environmental factors, the correlation analysis of primary data and, moreover, the covariance analysis of linear regressions have been executed.

## 4. DISCUSSION

Covariance analysis (ANCOVA) of primary data could be revealed significant differences in the values of the intercepts beside statistical constancy of the slopes. The state-dependent regressions of POD and PPO activities, the parameters of which are reported in *Table 1*, are depicted in *Figures 1a* and *1b*.

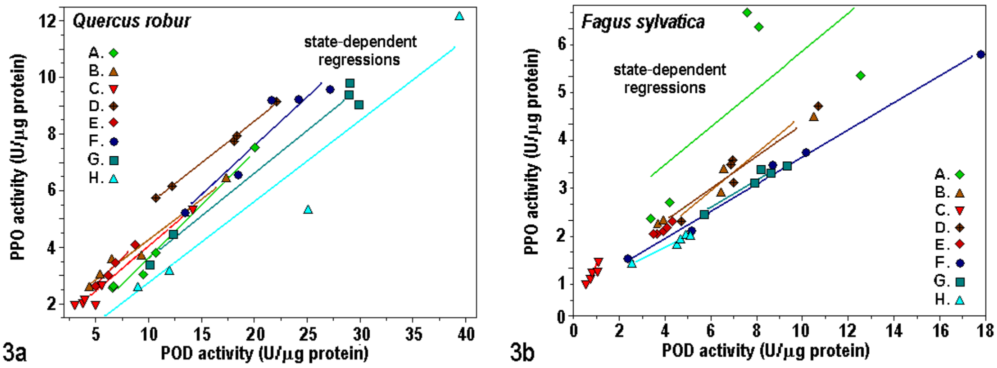


*Figure 1. State-dependent regressions of POD and PPO activities in the vegetation period of Pendunculate oak (1a) and beech trees (1b)*

Despite the absence of relevant deviation between the means, the physiological states with statistically various regressions are treated as distinguishable states from each other. The regression straight lines of the samplings are the estimations of the relationship of state-dependent correlation (*equation 1*). An alternative estimation can also be obtained by substituting the means ($M_{POD}$, $M_{PPO}$) and the empirical standard deviations ($SD_{POD}$, $SD_{PPO}$) for the expected values ($\mu_{POD}$, $\mu_{PPO}$) and the theoretical standard deviations ($\sigma_{POD}$, $\sigma_{PPO}$) in equation 1, respectively. The predictive results of the slopes and intercepts of theoretical equation are reported in *Table 3*. The strong correlations of different estimations confirm the power of the state-dependent correlation concept.

*Table 3. Estimations of the parameters of theoretical state-dependent correlation equation* (I. Prediction of slope and intercept with the means and standard deviations; II. Estimation by linear regression; * The data of May 15 are omitted.)

| Sampling | Slope $\left(\frac{\sigma_{PPO}}{\sigma_{POD}}\right)$ | | | | Intercept $\left(\mu_{PPO} - \frac{\sigma_{PPO}}{\sigma_{POD}}\mu_{POD}\right)$ | | | |
|---|---|---|---|---|---|---|---|---|
| | Beech | | Oak | | Beech | | Oak | |
| | I. | II. | I. | II. | I. | II. | I. | II. |
| 15 May | 0.575 | 0.395 | 0.379 | 0.376 | 0.296 | 1.586 | -0.663 | -0.632 |
| 30 May | 0.345 | 0.340 | 0.289 | 0.285 | 0.581 | 0.617 | 0.879 | 0.915 |
| 12 Jun | 0.904 | 0.887 | 0.315 | 0.315 | 0.100 | 0.116 | 0.406 | 0.408 |
| 26 Jun | 0.408 | 0.396 | 0.296 | 0.295 | 0.150 | 0.236 | 2.037 | 2.051 |
| 17 Jul | 0.348 | 0.336 | 0.398 | 0.395 | 0.461 | 0.508 | 0.134 | 0.156 |
| 31 Jul | 0.284 | 0.283 | 0.367 | 0.348 | 0.481 | 0.489 | -0.286 | 0.117 |
| 14 Aug | 0.300 | 0.281 | 0.304 | 0.302 | 0.52 | 0.591 | 0.032 | 0.070 |
| 28 Aug | 0.228 | 0.223 | 0.298 | 0.288 | 0.519 | 0.539 | -0.832 | -0.657 |
| R | 0.9997* | | 0.9894 | | 0.9885* | | 0.9897 | |

On the base of POD-PPO regressions studied by covariance analysis (ANCOVA; BUCHAN 2000), no significant differences of regression slopes have been established within and between two tree species. The state-dependent regressions of POD-PPO correlation can be described with a significantly different common (total) regression. Among the intercepts of state-dependent regressions significantly different ones can be found. The coefficients of determination ($R^2$) were mainly above the value of 0.9 during the vegetation experiment, too. To reveal the relations between the statistical parameters of enzyme activities and state-dependent regression and the environmental factors, parametric (Pearson's R) and non-parametric (Spearman's rho) correlation analysis were used. The *Tables 4* and *5* contain the values of parametric and non-parametric correlation coefficients between various biological and environmental variables. The lower and upper triangle matrices in the tables involve the Spearman's rho and the Pearson's R coefficients of beech and oak trees.

*Table 4. Correlation matrice of statistical and environmental parameters of the beech tree.*
(*The data of the sampling at 15 May were omitted because of the weak fitting of the regression.)

| **Beech** | ρ | Spearman's rho | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R | | $M_{PPO}$ | $SD_{PPO}$ | $M_{POD}$ | $SD_{POD}$ | m | b | $R^2$ | T | GSR | RH | P |
| | $M_{PPO}$ | | **0.7143** | **0.8571** | **0.7857** | -0.0357 | 0.0357 | 0.0714 | **-0.6071** | -0.4144 | *0.2500* | 0.1429 |
| P | $SD_{PPO}$ | **0.7292** | | **0.8214** | **0.9643** | -0.0714 | 0.2143 | **0.7143** | **-0.8929** | -0.3604 | *0.0714* | 0.2143 |
| e | $M_{POD}$ | **0.9509** | **0.7383** | | **0.8571** | -0.3929 | 0.2500 | 0.3214 | **-0.6429** | -0.2523 | ***0.5357*** | 0.4286 |
| a | $SD_{POD}$ | **0.6822** | **0.9792** | **0.7506** | | -0.1786 | 0.3214 | **0.6071** | **-0.9286** | -0.2523 | *0.1786* | 0.3571 |
| r | m | -0.5978 | -0.3028 | -0.7159 | -0.3899 | | **-0.5714** | -0.0714 | 0.1429 | -0.3424 | **-0.9286** | **-0.8214** |
| s | b | 0.3827 | 0.1416 | 0.5013 | 0.2535 | **-0.8290** | | 0.0714 | -0.4286 | -0.0901 | 0.4286 | 0.3214 |
| o | $R^2$ | 0.1503 | **0.7435** | 0.2682 | **0.7964** | -0.0829 | 0.1234 | | -0.6786 | 0.0360 | -0.1429 | 0.2857 |
| n | T | **-0.7711** | **-0.5569** | **-0.7902** | **-0.5672** | 0.8775 | -0.7076 | -0.2625 | | 0.2883 | -0.0357 | -0.2857 |
| 's | GSR | -0.4574 | -0.3162 | -0.3723 | -0.2052 | -0.0769 | 0.1695 | -0.0888 | 0.2569 | | 0.1982 | 0.6667 |
| | RH | *0.4699* | *0.1233* | ***0.6520*** | *0.2425* | **-0.9322** | 0.7781 | -0.0286 | -0.7019 | 0.0455 | | **0.7143** |
| R | P | 0.2523 | 0.2335 | 0.4754 | 0.4019 | **-0.8231** | 0.8368 | 0.3109 | -0.5641 | 0.4443 | **0.8184** | |

($M_{PPO}$ , $M_{POD}$ : the means of PPO and POD activities; $SD_{PPO}$ , $SD_{POD}$ : empirical standard deviations; m : slope; b : intercept; $R^2$ ; coefficient of determination; T : temperature; GSR : global solar radiation; RH : relative humidity; P : air pressure)

*Table 5. Correlation matrice of statistical and environmental parameters of the oak tree.*

| **Oak** | ρ | Spearman's rho | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R | | $M_{PPO}$ | $SD_{PPO}$ | $M_{POD}$ | $SD_{POD}$ | m | b | $R^2$ | T | GSR | RH | P |
| | $M_{PPO}$ | | 0.3571 | **0.8810** | 0.5000 | -0.1667 | -0.1429 | -0.3810 | *-0.5476* | -0.1317 | **0.5476** | 0.4524 |
| P | $SD_{PPO}$ | 0.3133 | | **0.6667** | **0.9762** | -0.3095 | **-0.8095** | -0.3810 | *-0.1190* | -0.1796 | **0.6190** | 0.4286 |
| e | $M_{POD}$ | **0.9161** | **0.6481** | | **0.7619** | -0.2143 | -0.4524 | -0.4524 | *-0.3333* | -0.0838 | **0.8095** | 0.5714 |
| a | $SD_{POD}$ | 0.2955 | **0.9878** | **0.6196** | | -0.3571 | -0.7619 | -0.4048 | *-0.2381* | -0.2515 | **0.6667** | 0.4524 |
| r | m | -0.2848 | -0.4729 | -0.3346 | -0.5779 | | -0.2143 | 0.1429 | 0.4286 | 0.2755 | 0.0952 | 0.2381 |
| s | b | 0.2691 | **-0.5291** | -0.0763 | -0.4340 | -0.3839 | | 0.3810 | -0.2143 | -0.1198 | -0.7143 | **-0.6429** |
| o | $R^2$ | -0.4429 | -0.3724 | -0.5335 | -0.3123 | -0.0206 | 0.3472 | | 0.4762 | -0.5509 | -0.5476 | **-0.8571** |
| n | T | *-0.4684* | *-0.2513* | *-0.4598* | *-0.2360* | 0.1505 | -0.0579 | 0.4025 | | 0.2994 | 0.0714 | -0.1429 |
| 's | GSR | -0.2695 | -0.2439 | -0.2405 | -0.2309 | 0.4486 | -0.2215 | -0.2774 | 0.2967 | | 0.2874 | 0.5988 |
| | RH | **0.5626** | **0.6096** | **0.7377** | **0.5875** | -0.0601 | -0.3321 | -0.4518 | -0.6859 | 0.0610 | | **0.8095** |
| R | P | 0.2978 | 0.4164 | 0.4852 | 0.3555 | 0.2645 | **-0.5853** | **-0.7329** | -0.5546 | 0.4000 | **0.8182** | |

($M_{PPO}$ , $M_{POD}$ : the means of PPO and POD activities; $SD_{PPO}$ , $SD_{POD}$ : empirical standard deviations; m : slope; b : intercept; $R^2$ ; coefficient of determination; T : temperature; GSR : global solar radiation; RH : relative humidity; P : air pressure)

The numbers in bold in the *Tables 4* and *5* highlight the variables pairs the correlation coefficients of which are bigger in their absolute values than 0.5 (abs(R) > 0.5 and abs(ρ) > 0.5). In these cases, the parametric and the non-parametric correlations render the existence of the relationships of the variables. The correlations of the temperature, the global solar radiation, the relative humidity and the air pressure to the statistical parameters of enzyme activities provide the coefficients with the same signs but different values. The means and standard deviations of PPO and POD show more relevant relations to the temperature in the case of the beech tree, while their stronger interdependences with relative humidity can be found at the oak tree. More relevant influence of the relative humidity just appears at the beech POD mean. The negative correlations of PPO and POD activities with the air temperature at forest trees are new results. Thus, during climatic alteration, the enhancement of the oxidative stress in the leaves of these forest trees can be rendered. The deviations experienced in the effect of temperature and relative humidity on the foliages are in harmony the deviations in environmental conditions of their habitats. Furthermore, physiological origin of the oak decline under summer droughts in many regions in Europe (DOLEZAL et al. 2010) can also be explained by the concept of state-dependent correlation mentioned before. The state-dependent correlation of PPO and POD activities can be regarded the indicator of regulated interaction between the trees and their altering environment.

## 5. CONCLUSIONS

The PPO and POD activities in the beech leaves are rather sensitive to temperature while those in the beech foliage rather correlate to relative humidity. The linear regressions of PPO-POD activities belonging to various environmental conditions of the samplings are the specific cases of a general PPO-POD state dependent correlation. Covariance analysis (ANCOVA) of state-dependent regressions suggests significant straight lines with different intercepts and undistinguishable slopes. Based on this ANCOVA, the centre points of the regressions, that is, the pairs of PPO and POD activity means represent distinguishable physiological states, too. In this way, the PPO-POD state-dependent regression can be considered an alternative indicator of physiological state of the forest trees. By applying the correlation analysis of the biochemical variables (e.g. PPO and POD), statistical parameters and environmental factors, extra information can be revealed about the interactions between the tree foliages and their environment. The temperature affects the PPO and POD activities in negative tendency while the relative humidity is in positive correlation to them.

**Acknowledgements:** This work was supported by a grant from the TÁMOP 4.2.1.B-09/1/KONV-2010-0006 research project.

### References

BRADFORD, M. M. (1976): A rapid sensitive method for the quantisation of microgram quantities of protein utilising the principle of protein-dye binding. Anal Biochem 72: 248-254.

BUCHAN, I. E. (2000): The development of a statistical computer software. Resource for medical resource. Liverpool, UK.

DOLEZAL, J. – MAZUREK P. – KLIMESOVA, J. (2010): Oak decline in southern Moravia: the association between climate change and early and late wood formation in oaks. Preslia 82: 289-306.

DUNFORD, H.B. (1999): Heme Peroxidases. Wiley-VCH, New York.

FLURKEY, W.H. – Jen, J.J. (1978): Peroxidase and polyphenol oxidase activities in developing peaches. J Food Sci 43: 1826–1828.

GONG, C.R. – WANG, A.H. – WANG, S.F. (2006): Changes of polyphenols in Tobacco leaves during the flue-curing process and correlation analysis on some chemical components. Agr Sci China 5: 928-932.

KHOSRAVINEJAD, F. – HEYDARI, R. – FARBOODNIA, T. (2008): Antioxidant responses of two barley varieties to saline stress. Res J Bio Sci 3: 486-490.

MAYER, A.M. (2006): Polyphenol oxidases in plants and fungi: Going places? A review. Phytochemistry 67: 2318-2331.

NÉMETH, Zs.I., – SÁRDI, É., – STEFANOVITS-BÁNYAI, É. (2009a): State-dependent correlations of biochemical variables in plants. J Chemomet 23: 197-210.

NÉMETH, Zs.I. – POZSGAI-HARSÁNYI, M. – STEFANOVITS-BÁNYAI, É. – SÁRDI, É. (2009b): Characterization of plant stress syndrome by some correlation of biochemical variables. Cereal Res Commun Suppl S, 37: 141-144.

NÉMETH, Zs.I.,– POZSGAI-HARSÁNYI, M. – GÁLOS, B. – ALBERT, L. (2009c): Stress sensitivity of correlation between oxidative activities in plants. Acta Silv Lign Hung 5: 27-45.