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Chemometric investigations of the multidrug resistance in strains of the phytopathogenic fungus *Penicillium digitatum*

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OUTLINE

INTRODUCTION

mandarin

orange

orange

grapefruit

apple

lemon

Penicillium digitatum or the green mold: a cause of serious problems in agriculture and even in medicine (immunocompromized patients).
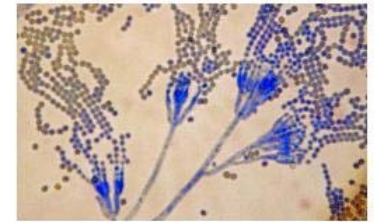
P. digitatum under microscope. The brush-like heads (Lat. *penicillus* = brush) have finger-like shape (Lat. *digitatum* = fingered) at their spore-producing ends.

The most frequent targets of *P. digitatum* are fruits, especially citric fruits.

GOAL

To propose novel chemometric approaches which can improve the use of bioassays data: identification and characterization of *P. digitatum* strains before applying adequate pesticides.
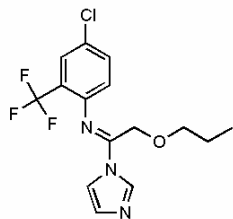
Literature data were used, with transformations when necessary.

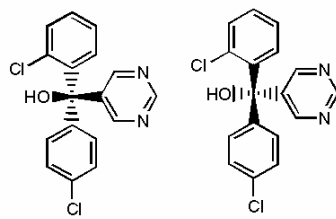The present work is contained in the following publications:

-R. Kiralj, M. M. C. Ferreira, *QSAR Comb. Sci.*, online since 17/07/2007

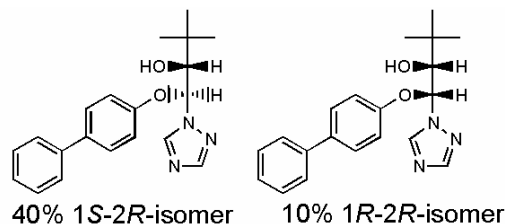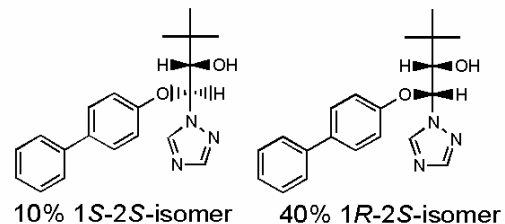-M. M C. Ferreira, R. Kiralj, *SAR QSAR Environ. Res.*, submitted.

STUDIED TOXICANTS

Triflumizole (I) or
(E)-4-chloro-α,α,α-trifluoro-
-N-(1-imidazol-1-yl-2-propoxy-
ethylidene)-o-toluidine,
a demethylation inhibitor

50% R-isomer   50% S-isomer
Fenarimol (II) or
(RS)-2,4'-dichloro-α-(pyrimidin-
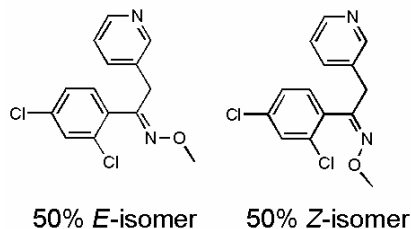-5-yl)-benzhydryl alcohol,
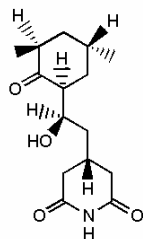a demethylation inhibitor

40% 1S-2R-isomer   10% 1R-2R-isomer

10% 1S-2S-isomer   40% 1R-2S-isomer
Bitertanol (III) or
(1RS,2RS)-1-(biphenyl-4-yloxy)-3,3-demethyl-
-1-(1H-1,2,4-triazol-1-yl)butan-2-ol,
a demethylation inhibitor

50% E-isomer   50% Z-isomer
Pyrifenox (IV) or
2',4'-dichloro-2-(3-pyridyl)aceto-
phenone-(EZ)-O-methyloxime,
a demethylation inhibitor

Cycloheximide (V) or
4-{(2R)-2-[(1S,3S,5S)-
-3,5-dimethyl-2-oxocyclo-
hexyl]--2-hydroxyethyl}-
piperidine-2,6-dione,
an antibiotic
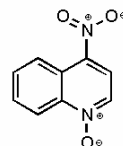
4NQO (VI) or
4-nitroquinoline-N-oxide,
a DNA intercalator
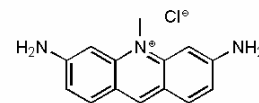
Acriflavine (VII) or
acriflavine chloride,
a DNA intercalator

Demethylation inhibitors (DMIs): **I-IV**

Antibiotic: **V**

DNA intercalators: **VI** and **VII**

EXPERIMENTAL DATA: RADIAL GROWTH DATA
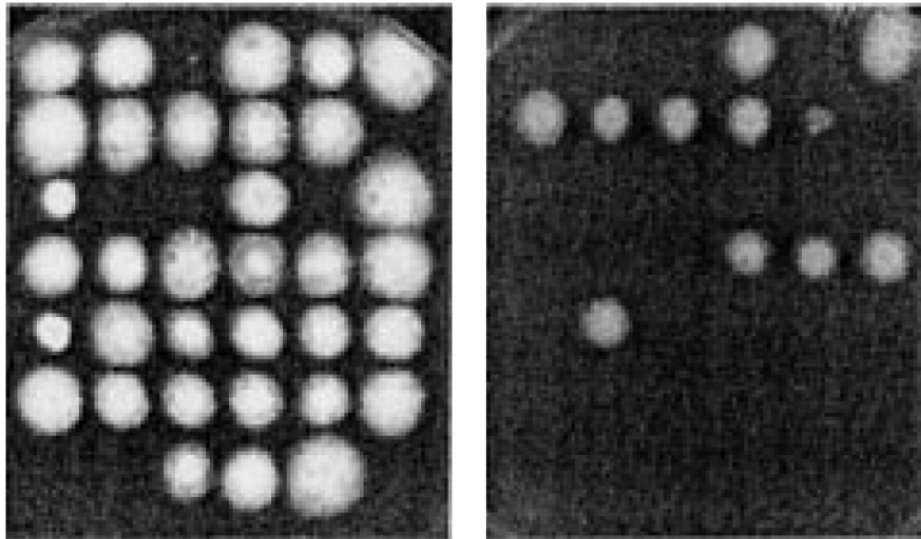
P. digitatum colonies
(1 strain = 1 colony):
-left: free growth
-right: inhibited growth
(Source: Hamamoto et al.,
Pest Manag. Sci. **57** (2001)
839-843.

Dose-response curves:
-no inhibition (C_0)
-50% inhibition (EC_{50},
Effective Concentration)
-100% inhibition (MIC,
Minimal Inhibitory
Concentration)

DATA SETS

<u>A</u>: $pECr_{50}$ values. EC_{50} transformed into $pEC_{50} = -\log(EC_{50}/\text{mol dm}^{-3})$ and then into $pECr_{50} = pE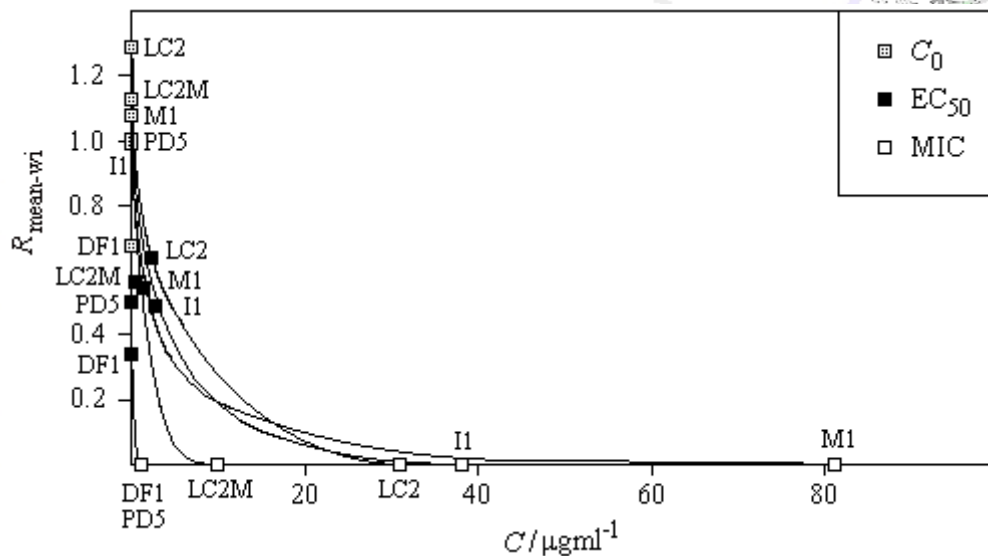C_{50}/pEC_{50}(PD5)$ where PD5 is the standard strain. Matrix $\mathbf{X}(6\times7)$, rows: strains, columns: toxicants

<u>B</u>: 8 Morphological descriptors of fungal colonies (35 strains): based on radii, circumferences and surface areas of the colonies from free growth and inhibited growth. Matrix $\mathbf{X}(35,8)$, rows: strains, columns: descriptors. Dependent variable $\mathbf{y}$: a genome descriptor PCR related to fungal resistance (production of the CYP51 protein).

<u>C</u>: 8 selected descriptors from a set of 6 genome descriptors related to fungal resistance (production of proteins CYP51 and PMR1) and 12 products of these descriptors with two molecular descriptors of toxicants. Matrix $\mathbf{X}(86,8)$, rows: strain-toxicant-experiment combinations, columns: selected descriptors. Dependent variable y: pEC_{50} values.

DATA SET <u>A</u>: PCA ANALYSIS
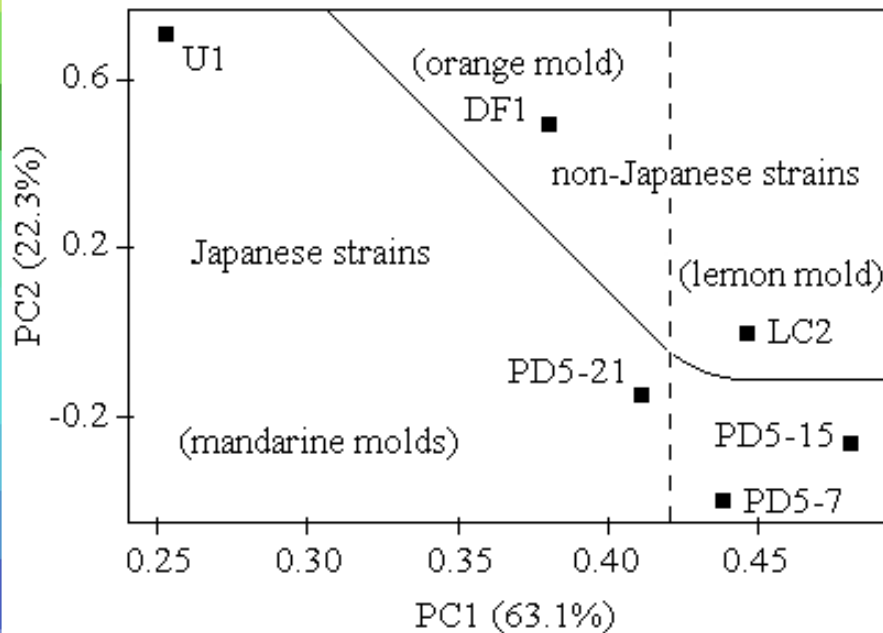
P. digitatum strains:
-resistant (DMI-R)
-moderately resistant (DMI-M)
-sensitive (DMI-S)
with respect to DMIs

Principal component analysis:
-autoscalled matrix **X**(6x7)
-PC1&PC2: 85% total variance

PC1-PC2 loadings plot. Strains characterization:

-resistance along PC1: sensitive (DMI-S) are left and resistant (DMI-R &DMI-M) right to the dashed line;

-diagonal curve: origin - Japanese and non-Japanese strains are separated; target fruits - mandarine molds and other molds are also separated.

DATA SET B: HCA AND PCA ANALYSES

Solid squares: external validation set for PLS
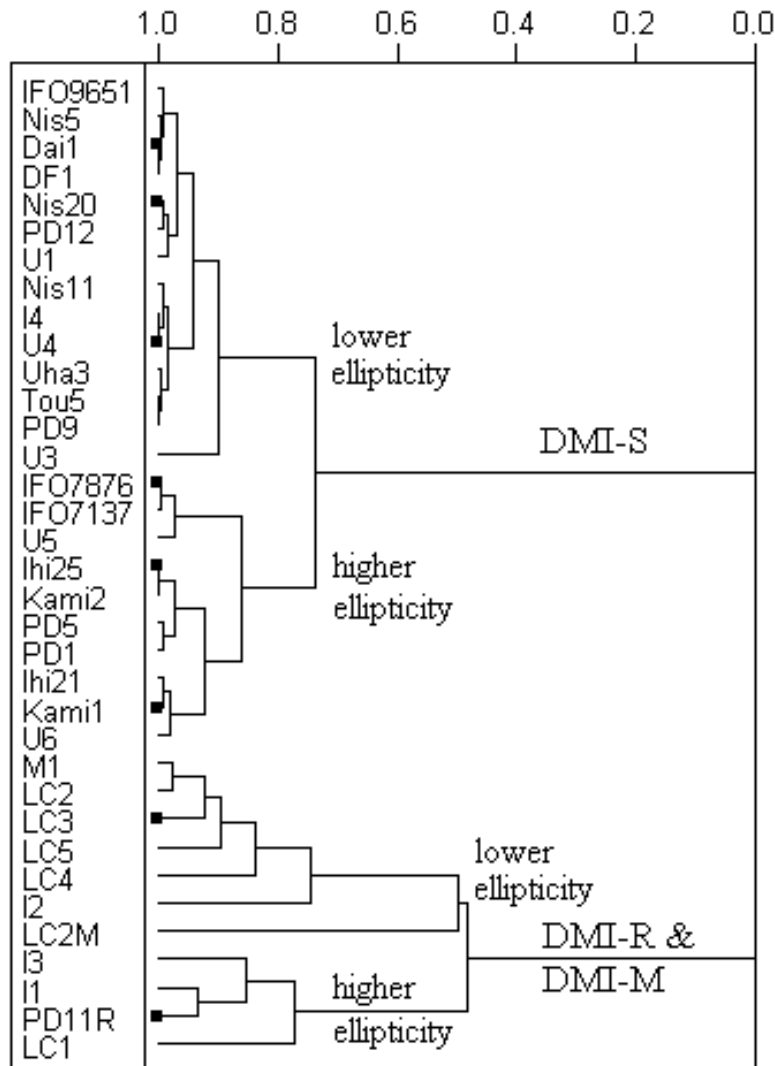
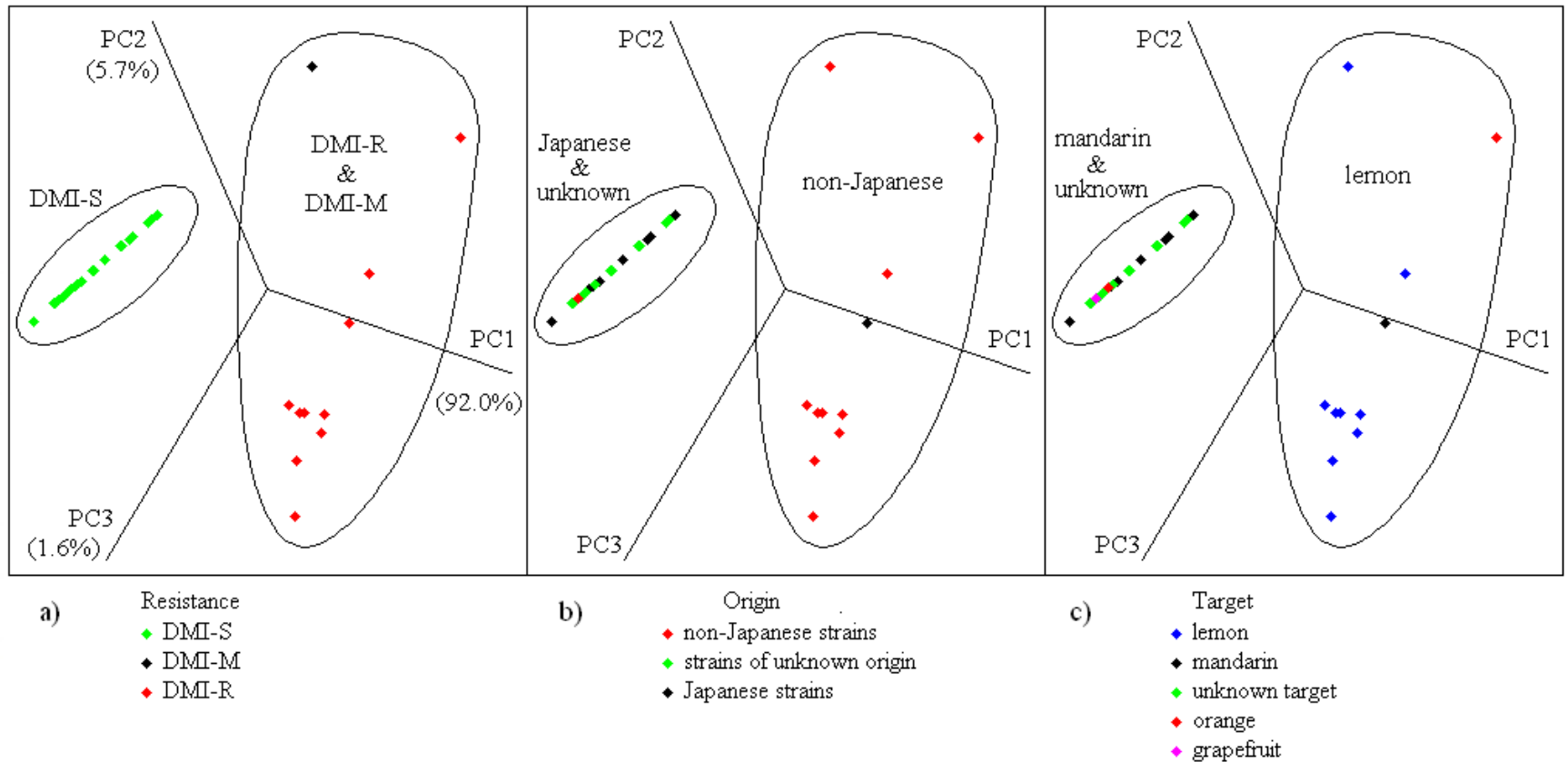Hierarchical cluster analysis:
-autoscalled matrix **X**(35x8)
-complete linkage

Clustering patterns:
-two clusters distinguishing sensitive (DMI-S) from resistant (DMI-R)&DMI-M) strains;
-two sub-clusters in each cluster: more round colonies (lower ellipticity) and more elongated colonies (higher ellipticity) when not treated with toxicants

Principal component analysis:
-autoscalled matrix **X**(35x8);   -PC1&PC2&PC3: 99% total variance.
Two cluster observed as in HCA, which distinguish reasonably well:
-resistance → resistant (DMIR&DMI-M) from sensitive (DMI-S) strains;
-origin → non-Japanese from Japanese&unknown strains;
-target fruits → lemon molds from mandarin&unknown molds.

DATA SET B: PLS REGRESSION (35 samples=strains)

The **y** variable:
PCR – a genome variable

The model's basic statistics:

2 PCs (97.6%)

Leave-one-out crossvalidation:
SEV = 0.028, **Q^2 = 0.991**

Prediction:
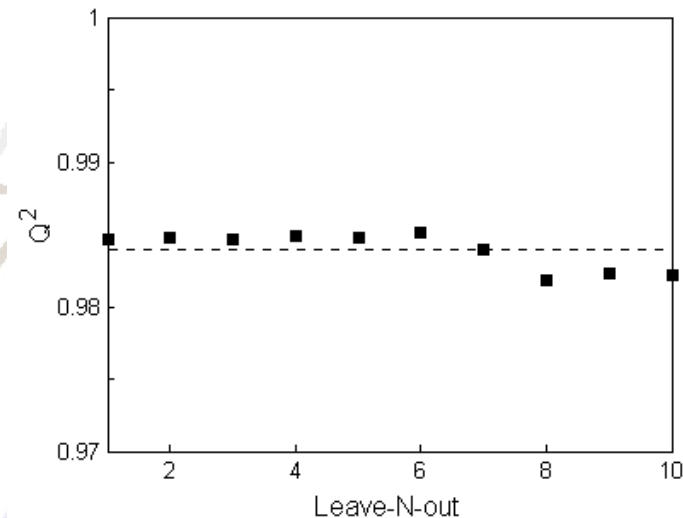SEP = 0.023, **R^2 = 0.985**

Relative errors:
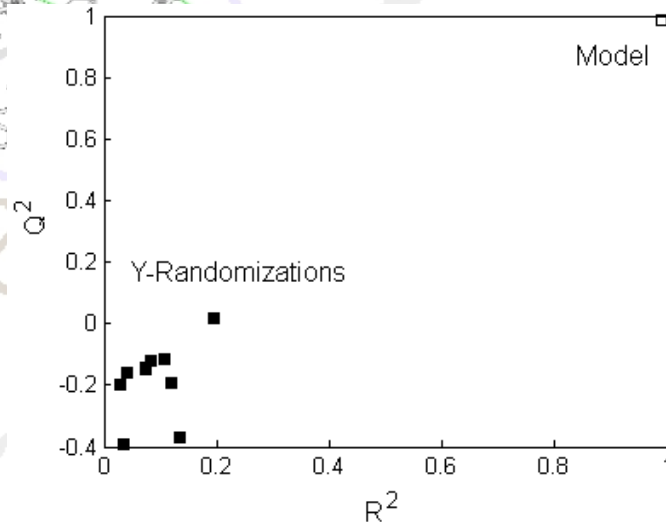-mean: 4.1%
-max.: 21.6% (DMI-M)
-samples with >10%: 1

External validation (8 samples in external validation set):
SEV = 0.030, SEP = 0.025
Q^2 = 0.982, **R^2 = 0.990**

Mean Q^2 is high.

No chance correlation.

DATA SET C: PLS REGRESSION (86 samples, 22 strains)

The **y** variable:
pEC$_{50}$ – inhibited radial growth

The model's basic statistics:

5 PCs (96.8%)

Leave-one-out crossvalidation:
SEV = 0.286, **Q^2 = 0.851**

Prediction:
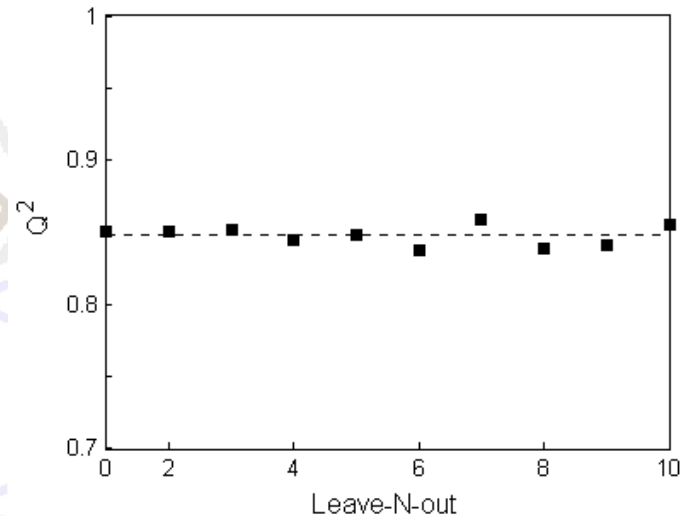SEP = 0.271, **R^2 = 0.874**

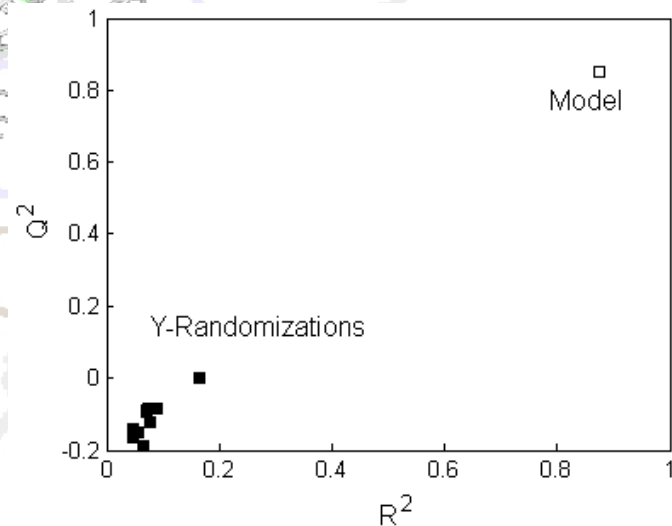Relative errors:
-mean: 3.3%
-max.: 13.3%
-samples with >10%: 2

External validation:
SEV = 0.305, SEP = 0.279
Q^2 = 0.841, **R^2 = 0.881**

Mean Q^2 is high.

No chance correlation.

CONCLUSIONS

Presented chemometric approaches to fungal growth data (EC_{50} and morphological data) are novel and promising procedures to identify and characterize *P. digitatum* strains in terms of their resistance to demethylation inhibitors, origin and target fruits.

Presented PLS regression models show direct quantitative relationships between genome structure related to the fungal resistance and the fungal growth data. By other words, *P. digitatum* strains can be well characterized knowing only one of the two types of data.