"Predicting fungal infection sensitivity of sepals in harvested tomatoes using Imaging Spectroscopy and Partial Least Squares Discriminant Analysis".

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Part 1: Introduction to the problem

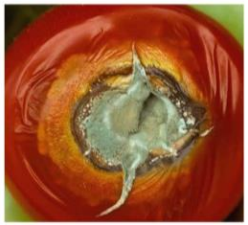
Problem definition

Source: Videos recorded by me under project number 6230217000

Problem definition

- Motivation: tomato - highly sensitive during harvest and post-harvest processes (transport, packaging and storage), susceptible to pathogenic fungi
- This leads to post-harvest losses, reaching up to 30% in some developing countries
- Early weakness of the sepals is not visible to the naked eye - no method exists of detecting this automatically prior to the infection

Aspergillus

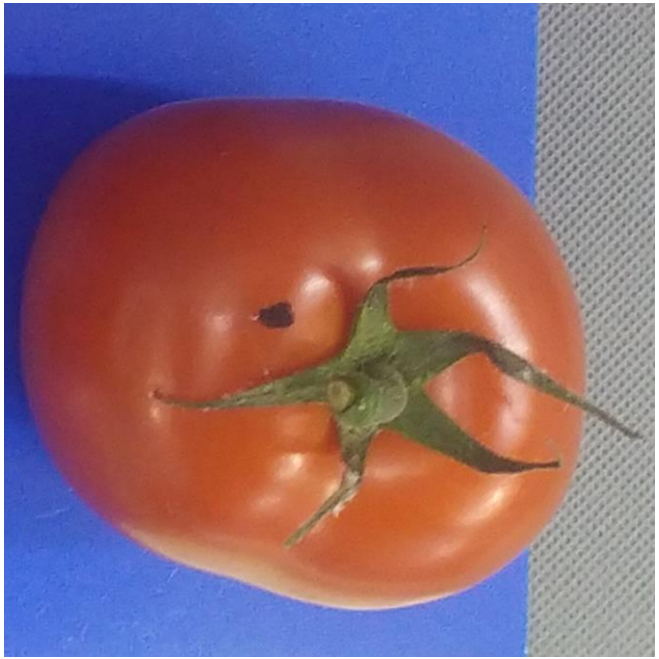


Penicillium

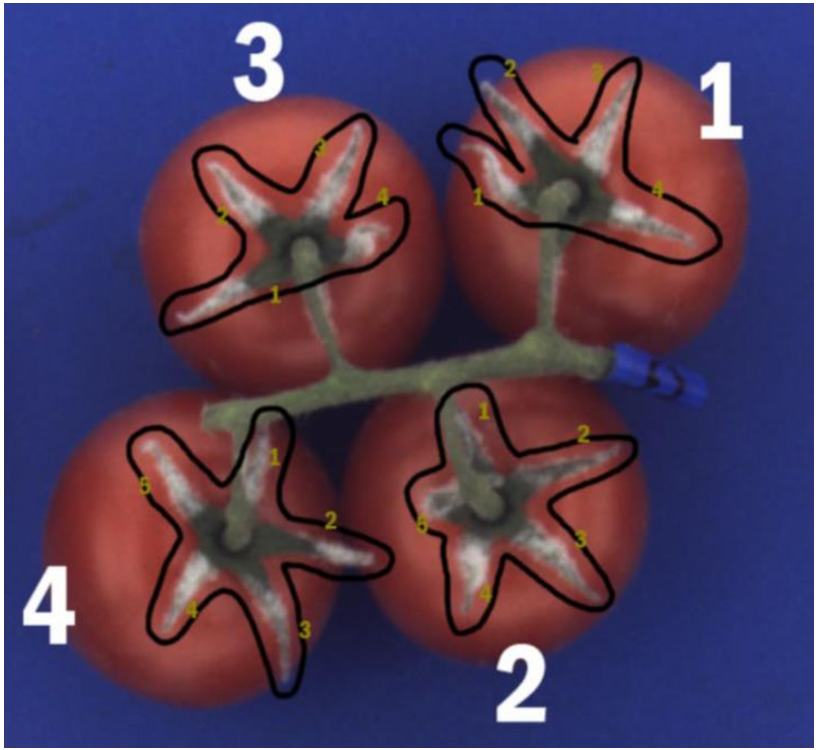
Mucor

????

It begins …



… and it gets worse (after 5 days)



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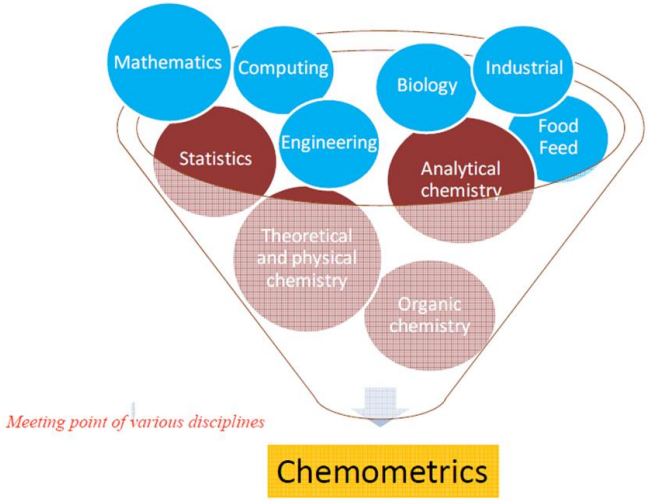
Objectives of this feasibility study

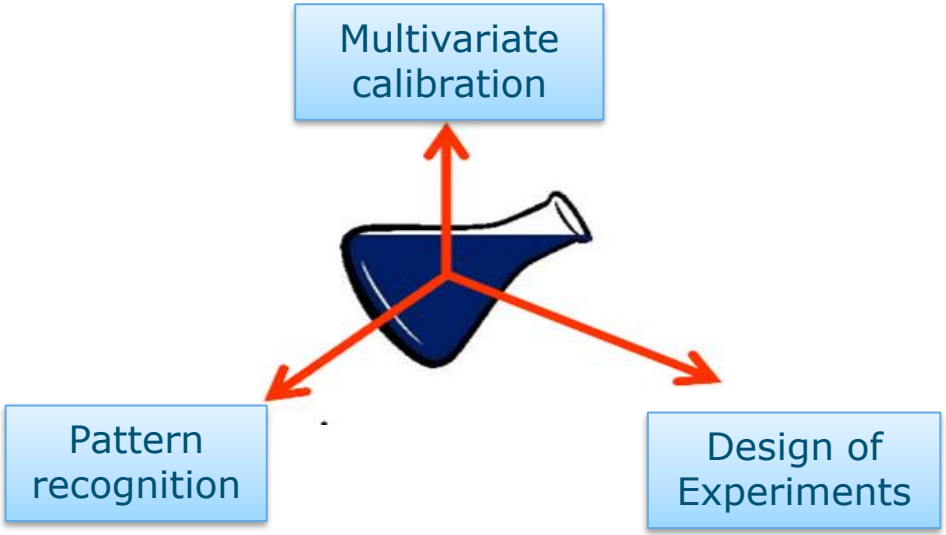
- Investigate if there is a correlation between the hyperspectral data captured at harvest and the fungal infection observed 3 and 4 days later by chemometrics using wrapping methods and PLSDA
- Calibrate and validate intravariety, intervariety and global model to grade the susceptibility to fungal infection, and to identify important wavelengths as input for the model

Part 2: Theory

Chemometrics

"Chemometrics is the chemical discipline that uses mathematical, statistical, and other methods employing formal logic to design or select optimal measurement procedures and experiments, and to provide maximum relevant chemical information by analyzing chemical data". **D. L. Massart (1941-2005)**





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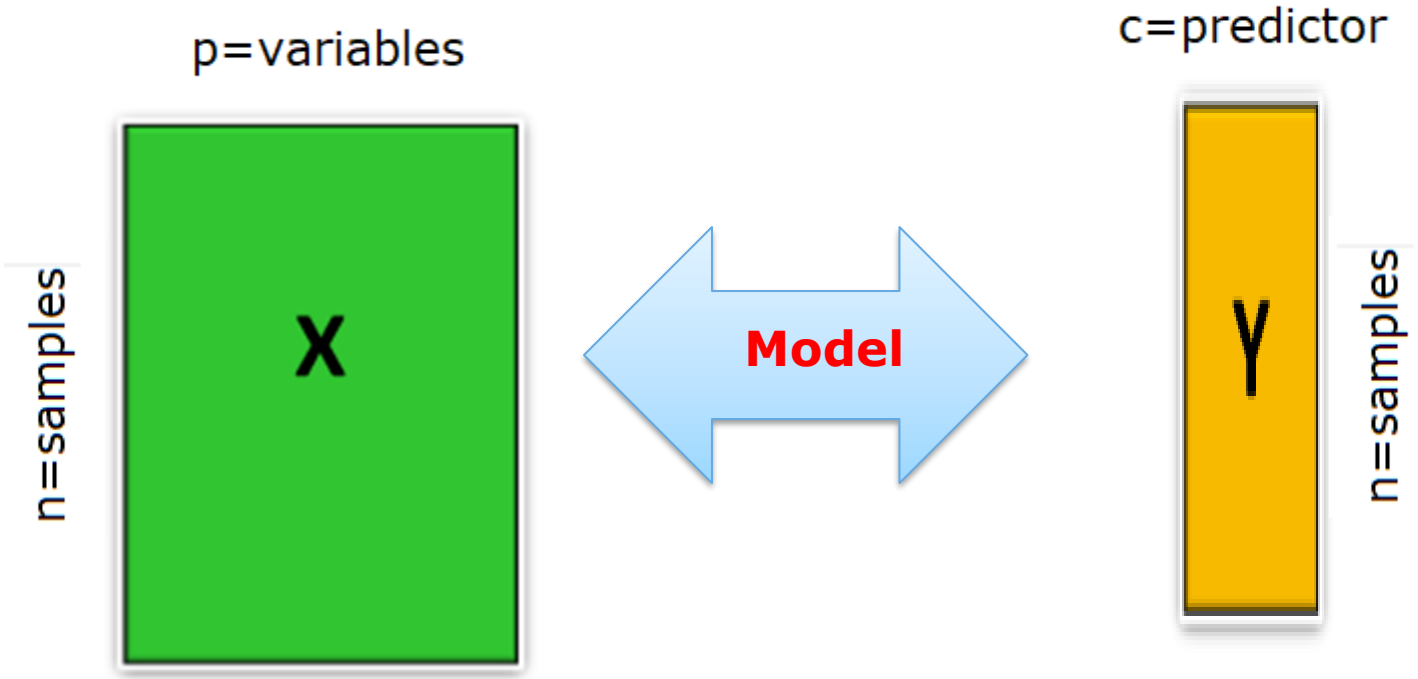
Why Chemometrics? Why Linear Algebra?

- NIR spectra are of high dimension
- The data are **highly correlated (collinearity problem)** and obscured by the presence of overlapping absorbances, harmonics, and **combination bands**
- Spectra are often complicated by light scattering and other physical effects
- Multivariate methods (chemometrics) are required to address these issues

- ***"Linear algebra is the language of chemometrics. To understand most chemometric techniques, a basic understanding of linear algebra is required."*** **(Wise and Gallagher, 1998)**

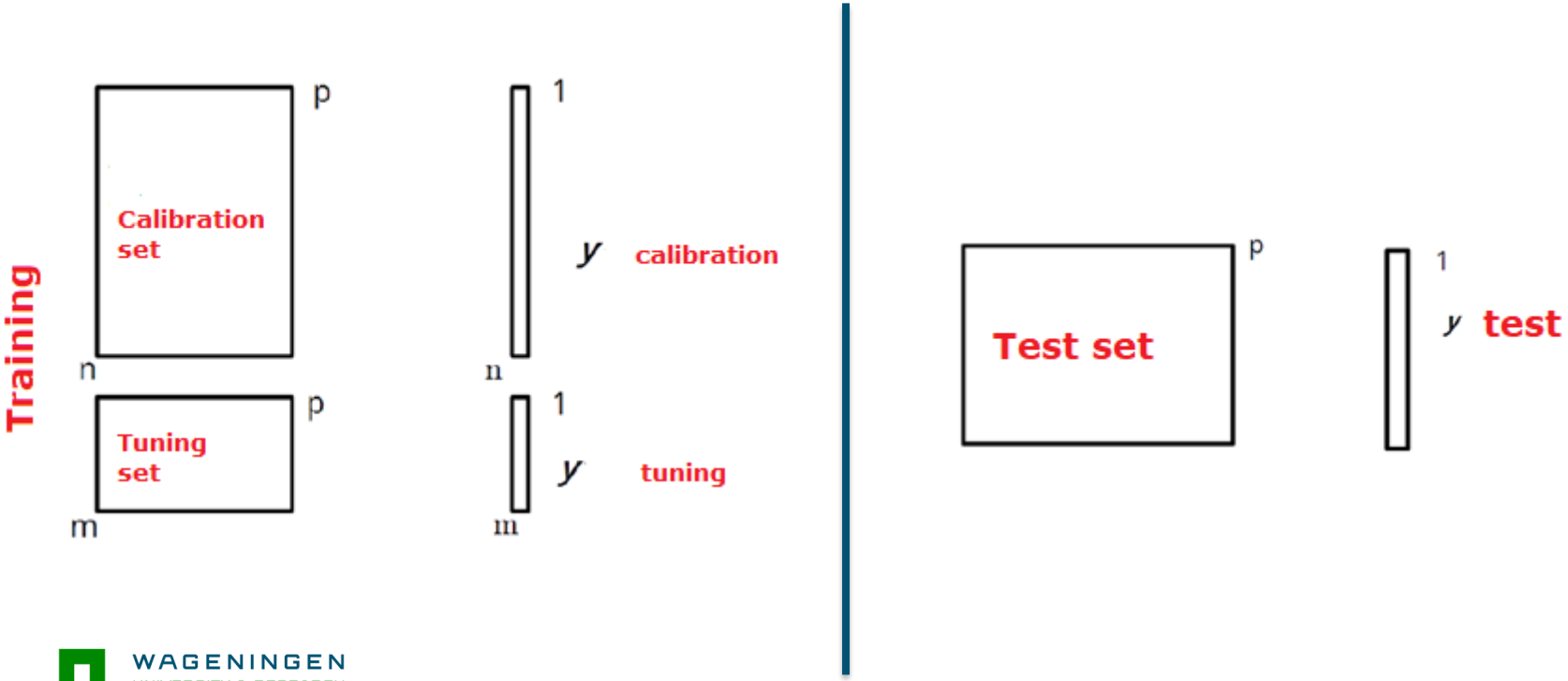
Multivariate analysis



The predictor variables are partially selective of the chemical properties

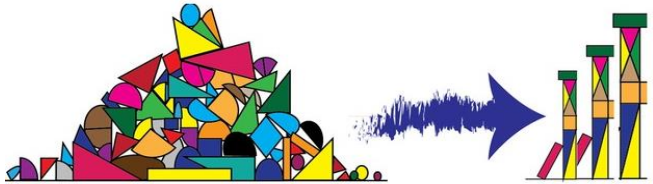
GOOD MODELING PRACTICES (GMP)

Data Set Preparation



Feature selection methods

Feature selection



Increasing the number of variables:

Introduces unnecessary NOISE for discrimination, especially if they are strongly correlated

Carries a risk of OVERFITTING the models

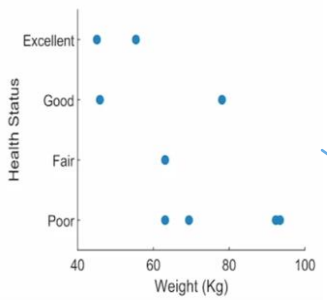
Using a simple model with few variables has a better chance of being generalized to a new sample than a model with hundreds of variables, which may fit the training set perfectly well but has limited generalization power

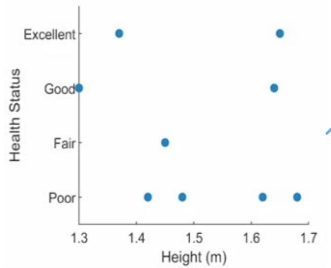
3 Approaches:
1. Variable Transformation and/or Selection
2. Discretization
3. Group Summary

Variable transformation

This involves applying an equation to existing variables to create a new feature

Example: Is it possible to accurately classify the health status of each individual from the original variables of age, height, weight and location?

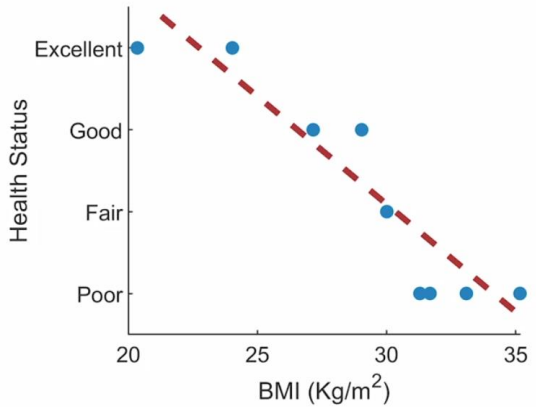




No clear correlation

$$BMI = \frac{\text{Weight (Kg)}}{\text{Height (m)}^2}$$

Correlation



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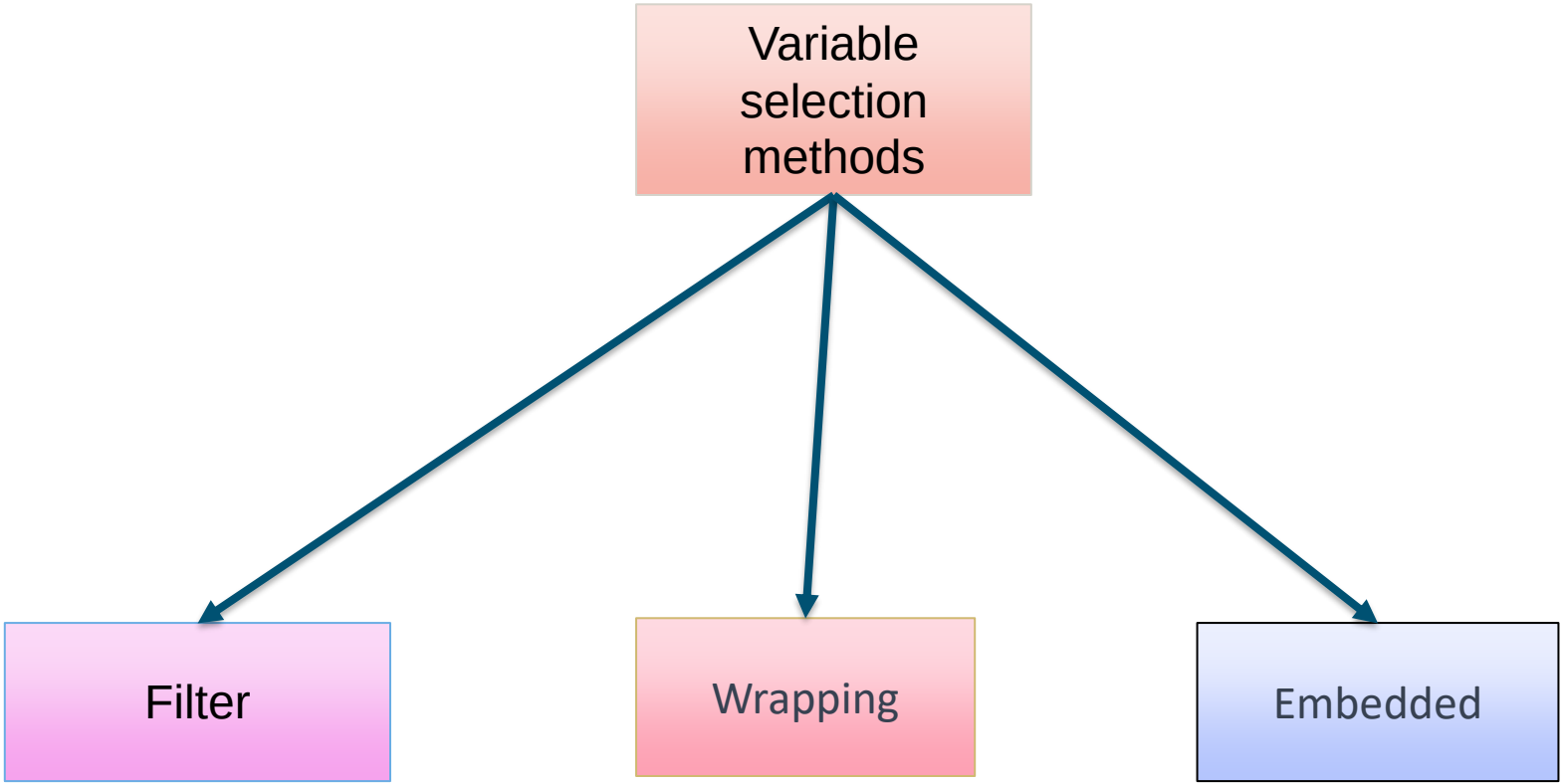
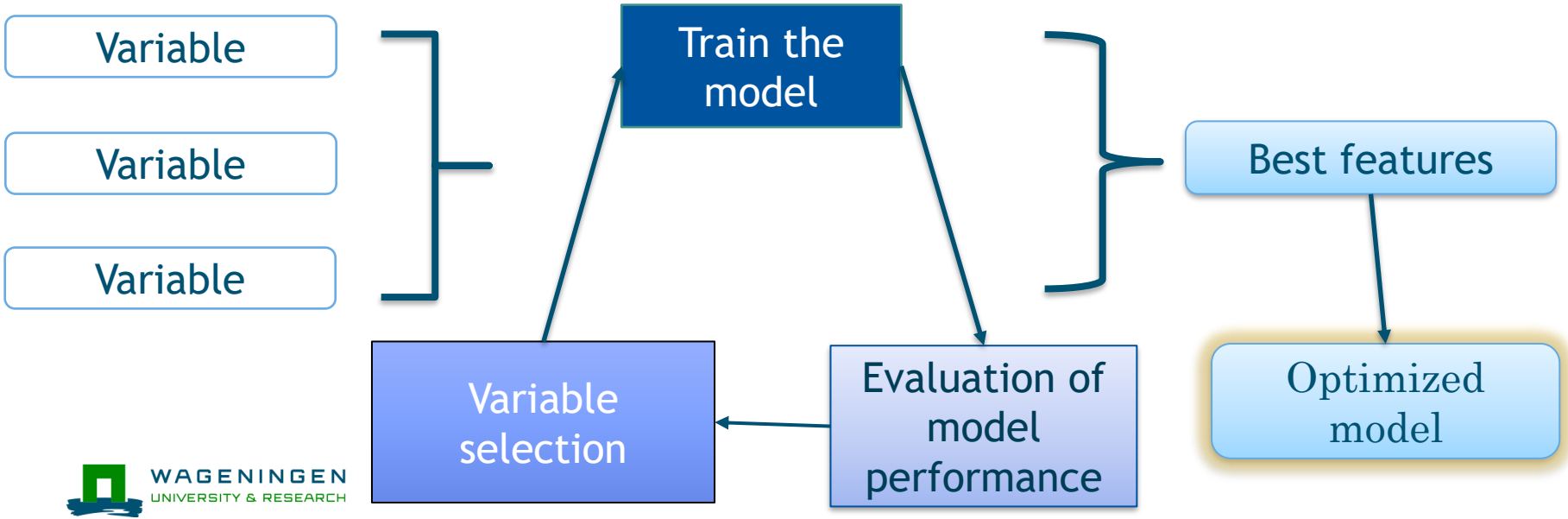

Variable selection methods
Filter
Wrapping
Embedded

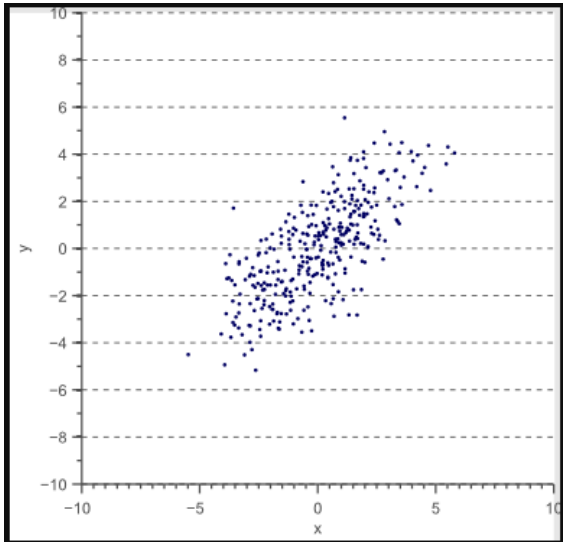
Wrapping methods

These variable selection methods depend on model performance. It is an iterative process of back and forth, where the chosen features are evaluated in relation to the final model performance in sequential stages.



Covariance

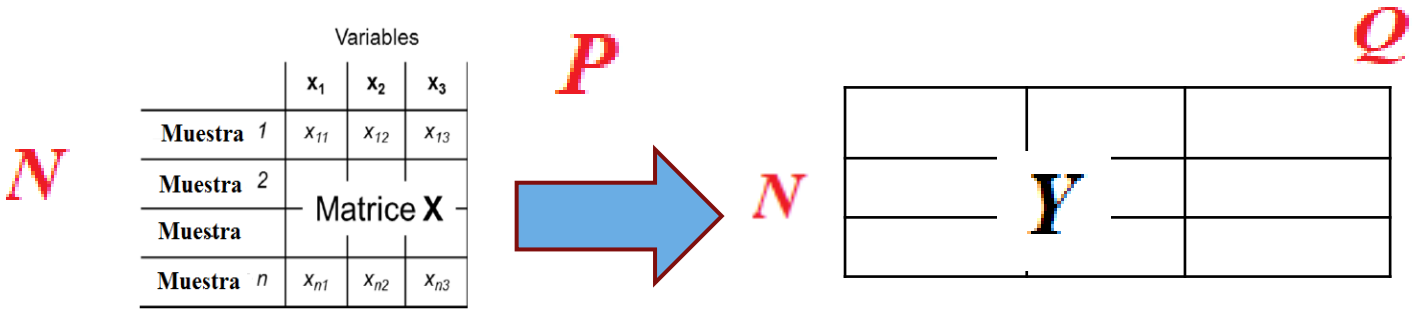
- Indicates the degree of joint variation between two random variables with respect to their means
- It can be used to understand the direction of the relationship between two variables
- The correlation coefficient is equal to the covariance divided by the product of the standard deviations of the variables



$$Cov(X,Y) = \frac{\sum_1^n (x_i - \bar{x})(y_i - \bar{y})}{n}$$

Wrapping method: Covariance selection (CovSel)

In each iteration, one variable **X** is selected on a criterion of the maximization of the covariances with **Y**



Once the variable with the highest covariance is isolated and selected, all other predictive factors and responses are orthogonalized with respect to it, and the process is repeated until the fixed number of variables has been selected

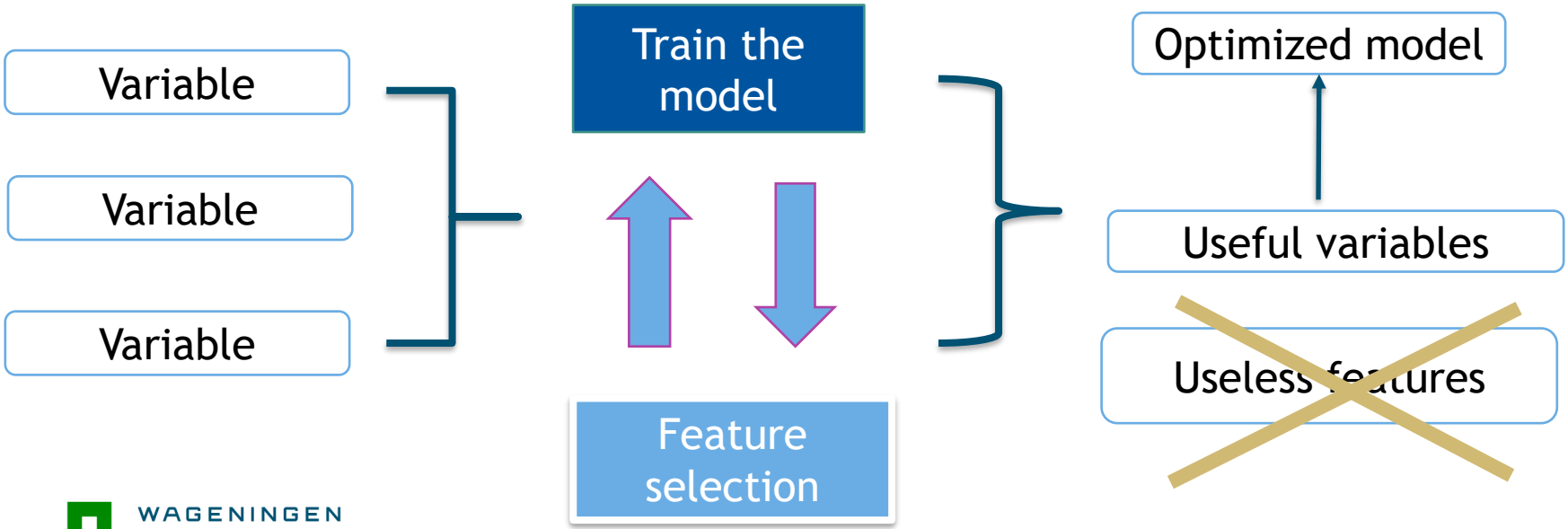
Covariance selection (CovSel)

The selected variables from X should have:

- **Good predictive power for Y**
- **The highest possible variability**

Embedded methods

They automatically perform feature selection as part of the model training
The result is a trained model that highlights the useful features and disregards the rest

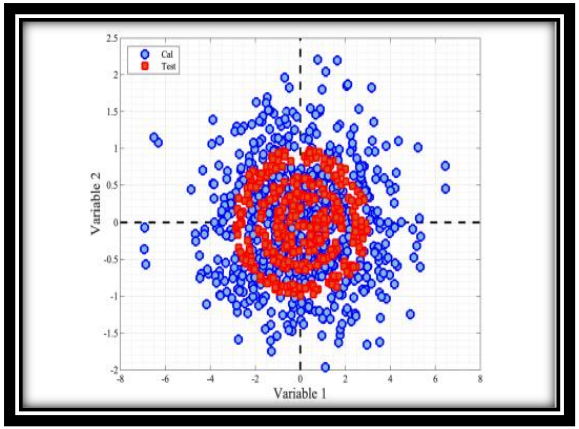


Cross validation and Data Split

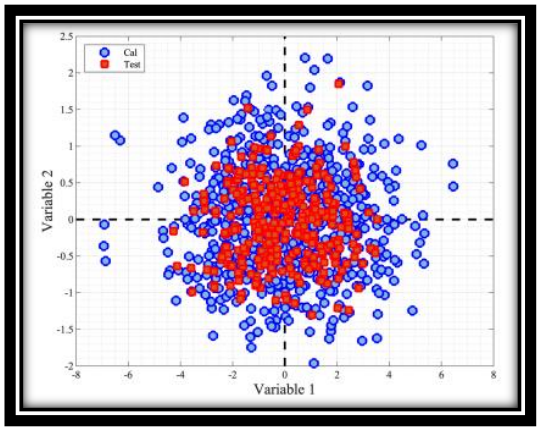
Some methods for the selection of Representative Learning and Test Sets

- Randomly
- Kernnand Stone
- Onion
- Duplex
- Reducennsamples

Onion



Kennard-Stone

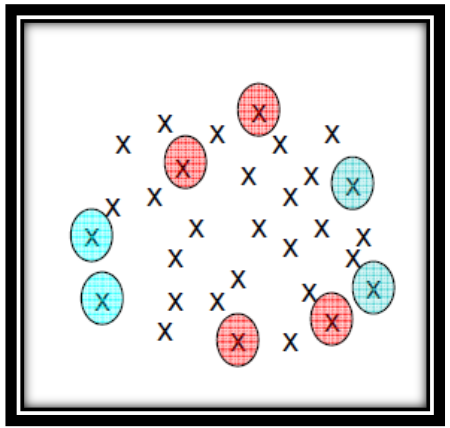


Source:https://eigenvector.com/wpcontent/uploads/2022/10/Onion_SampleSelection.pdf

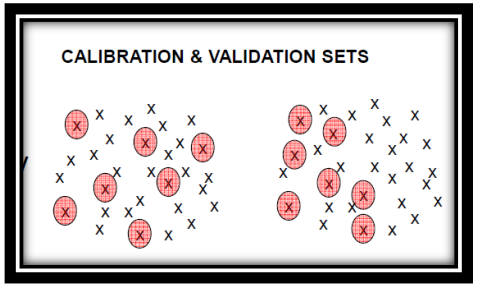
Some methods for the selection of Representative Learning and Test Sets

- Randomly
- Kernnand Stone
- Onion
- Duplex
- Reducennsamples

Duplex



Randomly

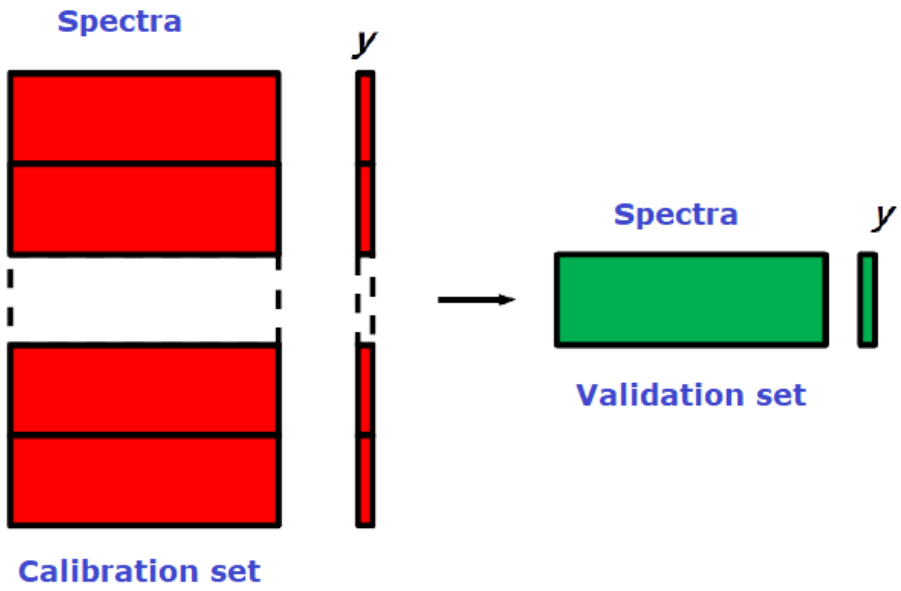


Source:https://eigenvector.com/wpcontent/uploads/2022/10/Onion_SampleSelection.pdf

Cross validation

Leave One Out: Over-estimates the predictive capacity of the model

Use only when data set has few samples



Discrimination (PLSDA)

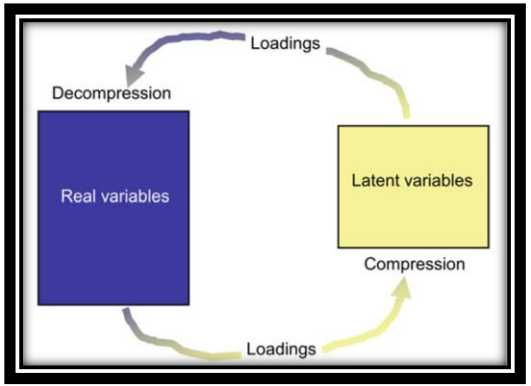
Partial Least Squares Discriminant Analysis

- Objective: To achieve a linear transformation that maps the data into a lower-dimensional space with the least possible error
- Supervised version of PCA
- In **PCA**, the transformation preserves (in its first principal component) the maximum possible variation in the original data
- In **PLS-DA**, the transformation preserves (in its first principal component) the maximum possible **covariance** between the original data and their labeling

Both can be described as iterative processes in which the error term is used to define the next principal component

Partial Least Squares Discriminant Analysis

- It consists of a classical PLS regression where the response variable is a category expressing the membership of samples in classes
- The relevant sources of data variability are modeled by the Latent Variables (LVs) which are linear combinations of the original variables



A fictitious matrix (Y) that records membership with 1s and 0s is combined with a spectral set (X), and PLS is implemented in the normal manner

$$y = \begin{pmatrix} 1 \\ 1 \\ 2 \\ 1 \\ 3 \\ 1 \\ 2 \\ 2 \\ 3 \end{pmatrix} \rightarrow Y = \begin{pmatrix} 1 & 0 & 0 \\ 1 & 0 & 0 \\ 0 & 1 & 0 \\ 1 & 0 & 0 \\ 0 & 0 & 1 \\ 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{pmatrix}$$

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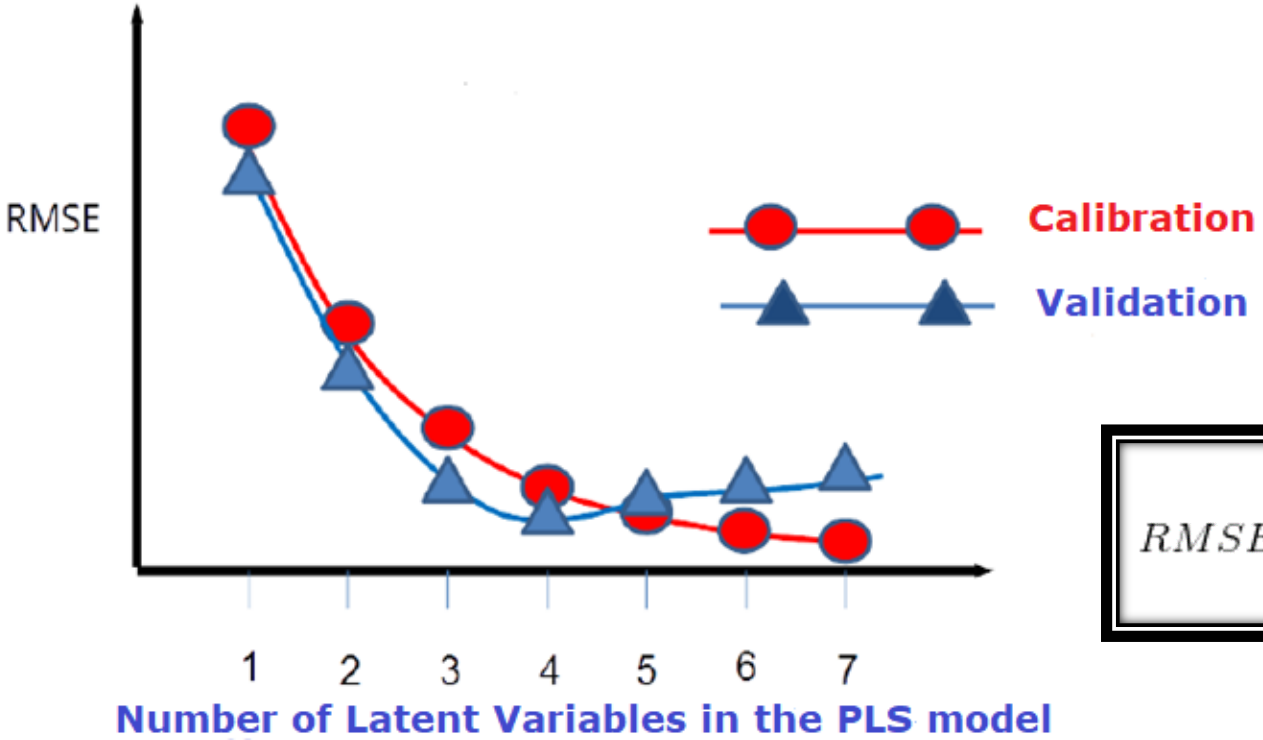
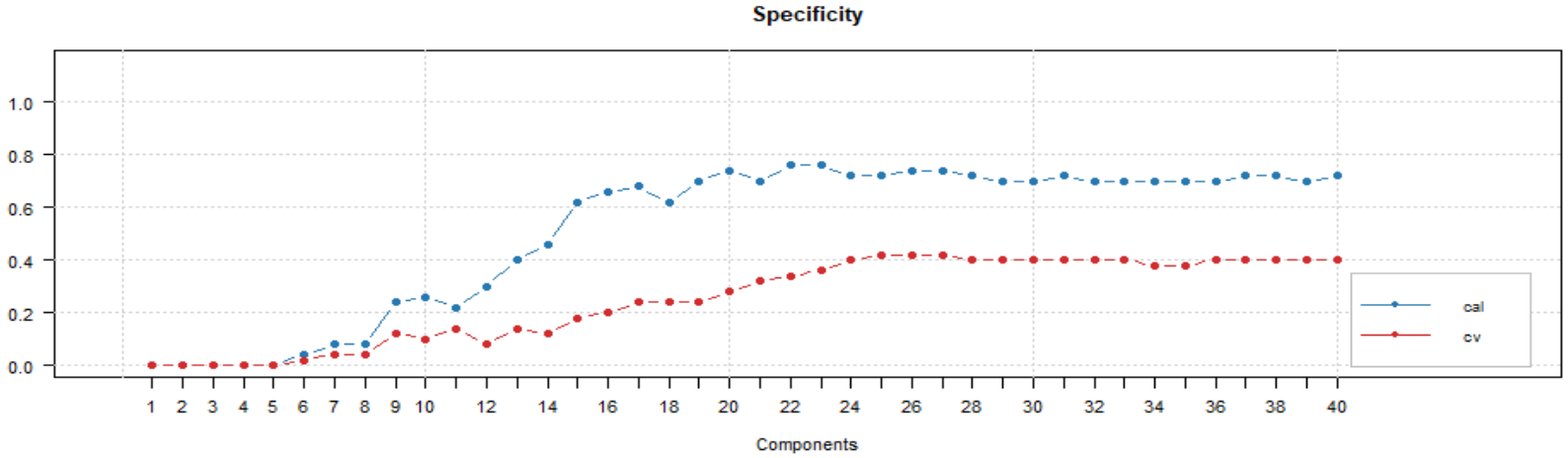
Partial Least Squares Discriminant Analysis

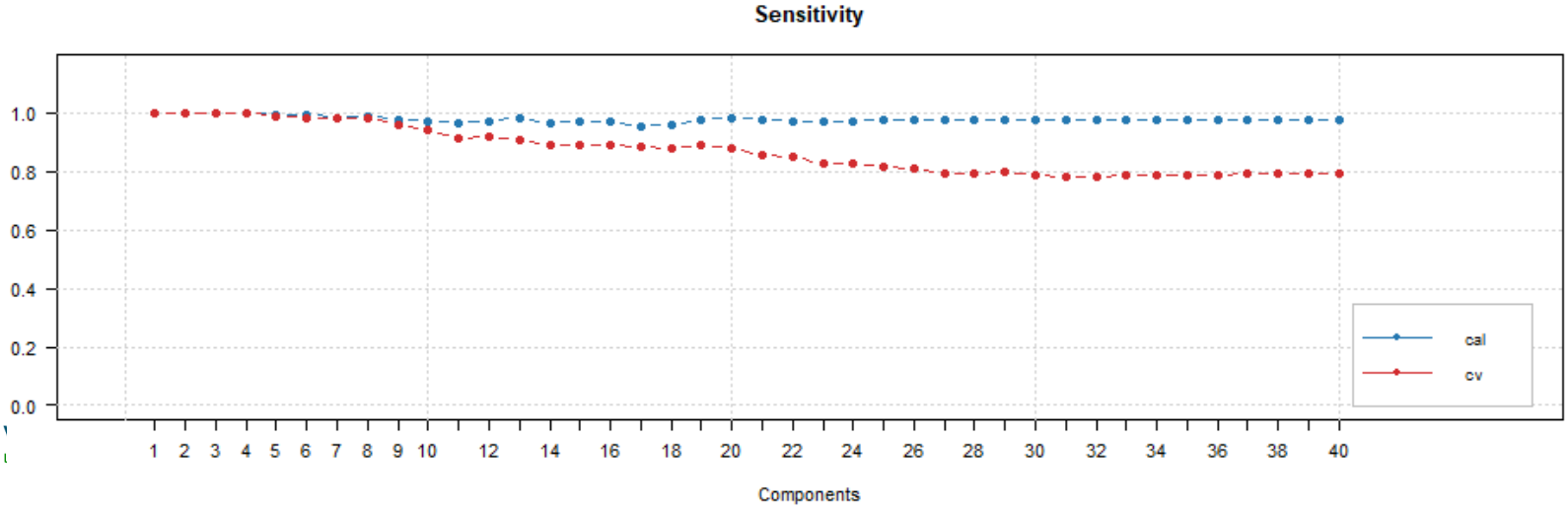
- PLS-DA provides estimated values for each sample and for each class.
- These values will not be exactly 1 or 0; however, if the calculated **y** is closer to 0, then the sample likely does not belong to that class, while a value closer to 1 would indicate the opposite
- To make a class assignment, a **threshold** can be defined for each class
- Thresholds are defined where false positives and false negatives are minimized

Study of Error as a Function of Dimensionality



$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}$$


Specificity
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40
Components
cal
cv


Sensitivity
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Components
cal
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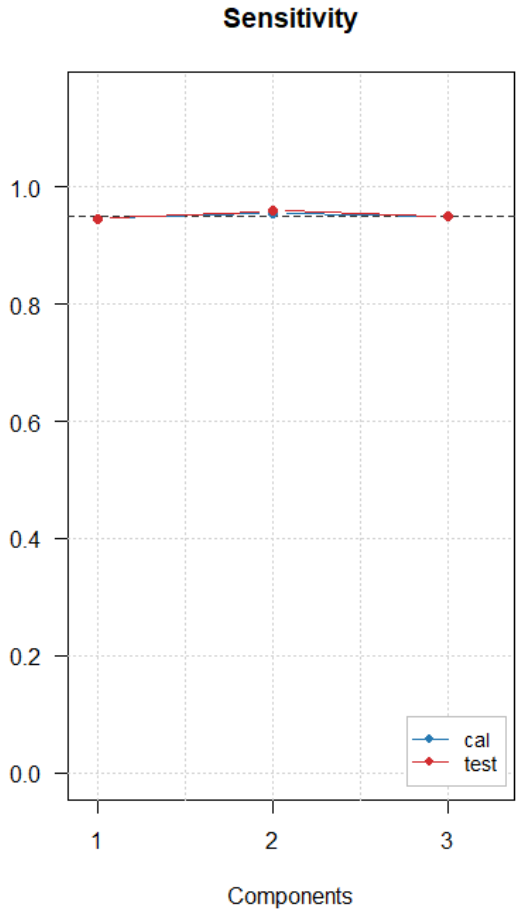
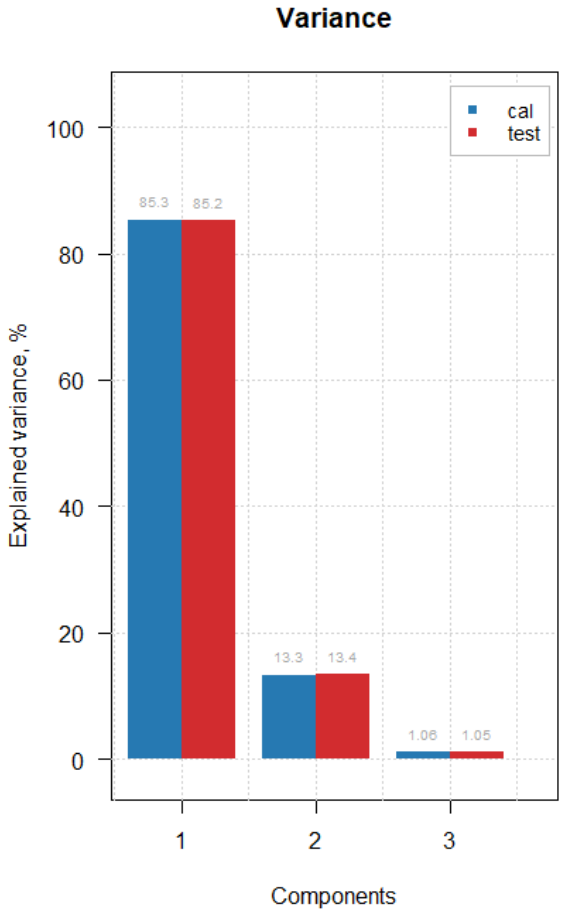

Sensitivity
1.0
0.8
0.6
0.4
0.2
0.0
cal
test
1
2
3
Components

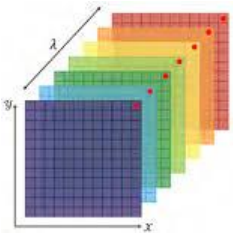

Variance
cal
test
100
80
60
40
20
0
Explained variance, %
85.3
85.2
13.3
13.4
1.06
1.05
1
2
3
Components

Materials and methods

Data description

Tomatoes origin

- The samples belong to 3 cultivars – Brioso, Cappricia and Provine
- They all come from different greenhouses
- Tomatoes were harvested fresh on the 9th and the 10th of May.
- They arrived at Phenomea Laboratory, in Wageningen on Tuesday 10th May



Data set

- NIR dataset: hyperspectral images (SPECIM FX17 camera) of the tomatoes from the beginning of the experiment
- Reference values from the last day of the experiment, when the fungi have infected most of the sepals

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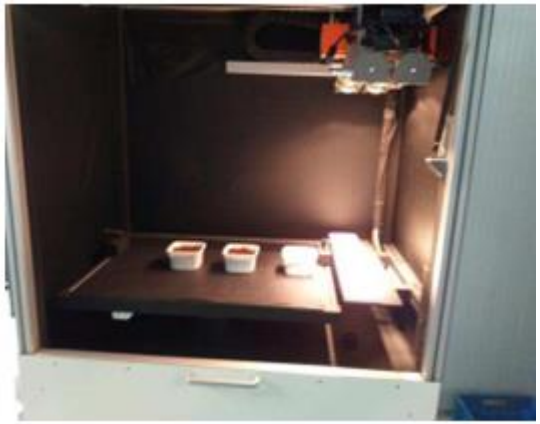
Experiment flow

1

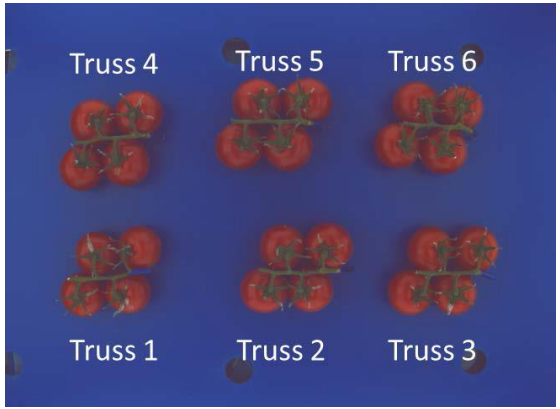


Hyperspectral imaging of tomato trusses using spectral cabinet*

2

Tomatoes in Hotbox - environment for controlled humidity and temperature Relative humidity 100%

3



Taking phone images in controlled environment

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*Source: Puneet Mishra, et al. All-in-one: A spectral imaging laboratory system for standardised automated image acquisition and real-time spectral model deployment, Analytica Chimica Acta, Volume 1190, 2022, 339235, ISSN 0003-2670,

General analysis steps

- Ground truth observations were made by experts on day three and four, comprised of severity scores from zero (no fungus) to four (severe infection).

- Ratings of the two days, and 3 experts were averaged
- NIR spectra of sepals extracted from Hyperspectral images
- Exploration of sepals at a pixel level by Principal Component Analysis
- Removal of outlier pixels
- Creation of qualitative models by chemometrics analysis using R studio version 4.2.1 (2022-06-23 ucrt)

Chemometrics

R version 4.2.1 (2022-06-23 ucrt)

1. Visualization of spectra
2. Exploration by PCA and outlier removal (at pixel and sepal level by tomato, by variety and by final dataset)
3. Preprocessing of raw NIR data
4. Data split 70/30 in a representative way for each class
5. Optimization of the number of important variables by CovSel algorithm
6. Optimization of the number of latent variables by PLSDA
7. Prediction using an independent test set
8. Evaluation of model according to usual classification parameters

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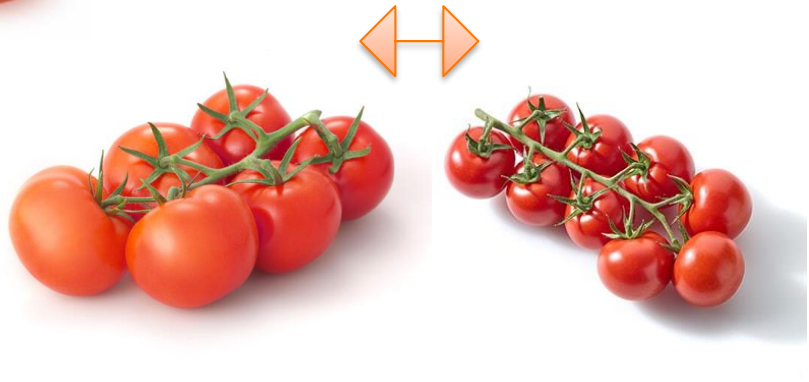
Classification design scenarios

Intravariety

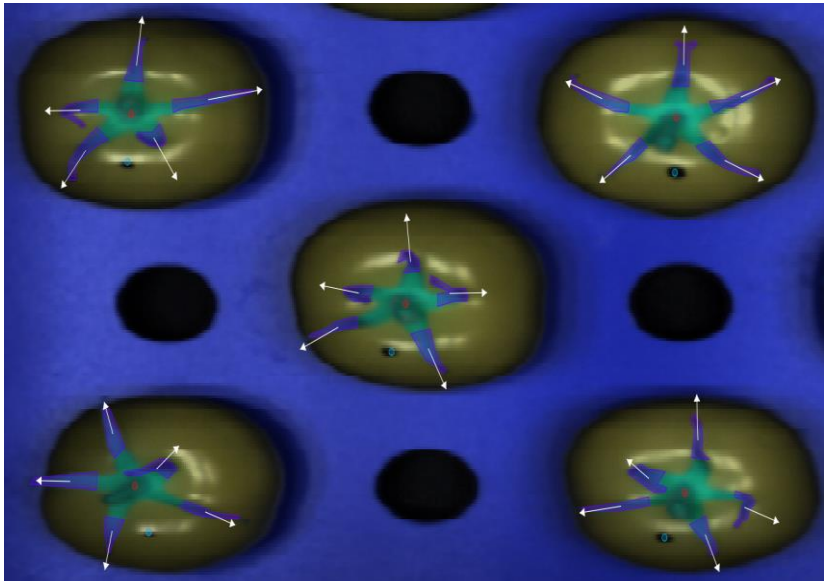
Global model

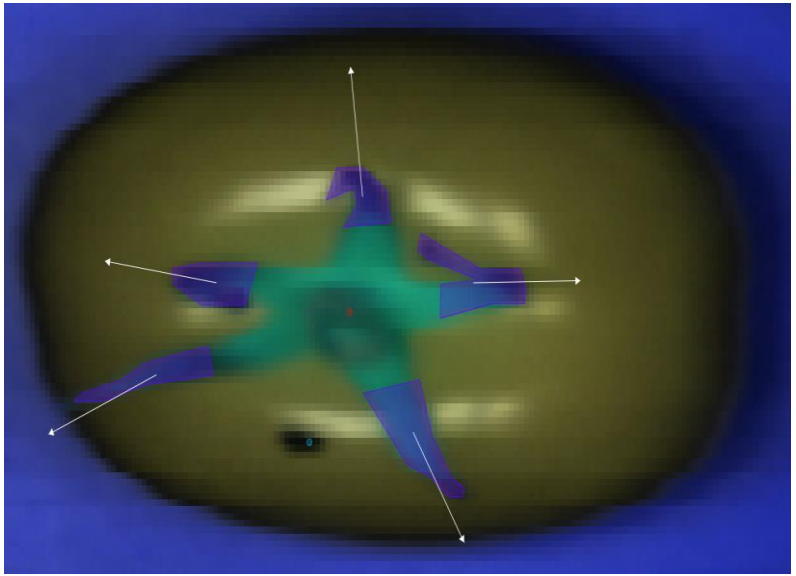
Intervariety



Results

Segmentation and extraction of NIR spectra

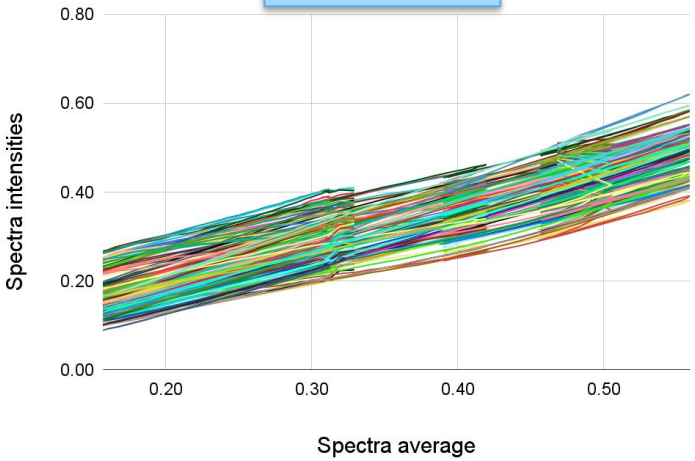




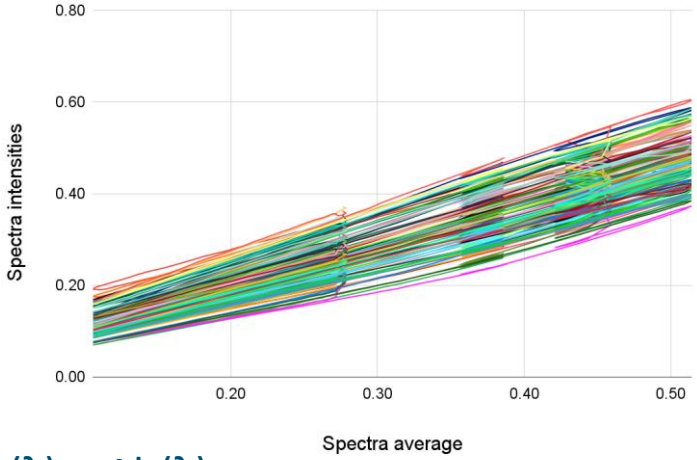
Villiers, Hendrik de
<hendrik.devilliers@wur.nl>

Part 3: Spectra pretreatments

Cappricia



Provine



$$A(\lambda) = k\varepsilon(\lambda)LC + Af(\lambda) + Ab(\lambda)$$

Where, A: Absorbance; λ: Wavelength; A(λ): Absorbance at a particular wavelength k: multiplicative effect; ε: Molar absorption coefficient (M^{-1} cm^{-1}); L: Optical path length (cm) C: Molar concentration; Af (λ). Additive effect at a particular wavelength caused by a certain number of photons escaping from the sensor; Ab(λ). Additive effect at a particular wavelength caused by random phenomena

Part 4: Sampling

Labelling scenarios:

1: 0/123, 2: 01/23, 3: 0.5/123

Variety	n	Healthy (Label 1)	Diseased (Label 1)	Healthy (Label 2)	Diseased (Label 2)	Healthy (Label 3)	Diseased (Label 3)
Cappriccia	163	139	24	85	78	117	46
Brioso	153	145	8	78	75	126	27
Provine	152	137	15	72	80	129	23

Data split was carried out in a representative way for each class 70/30, at a sepal level

All the sepals that belong to the same tomatoes were kept together

Part 5: Feature selection

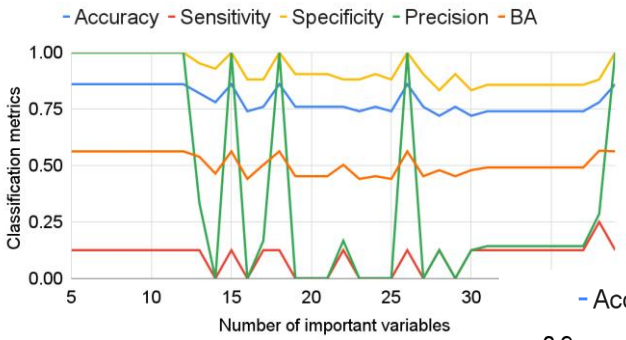
Most important variables for each variety

Label 2

Cappricia	937.33	944.25	951.16	965	1062.1	1083	1208.6	1320.9	1391.4	1426.7	1462.1	1561.4	1654.1	1704.1	1711.3
Provine	937.33	944.25	951.16	978.85	1089.9	1138.7	1201.6	1299.8	1370.2	1391.4	1440.8	1554.3	1654.1	1697	1718.4
Brioso	937.33	944.25	951.16	1089.9	1299.8	1391.4	1462.1	1589.9	1625.5	1661.2	1689.8	1697	1704.1	1711.3	1718.4
Global Model	937.33	944.25	958.08	1055.2	1089.9	1208.6	1278.7	1363.2	1405.5	1462.1	1589.9	1654.1	1704.1	1711.3	1718.4

Intravariety: Cappricia classification metrics according to different number of important variables as input for PLSDA

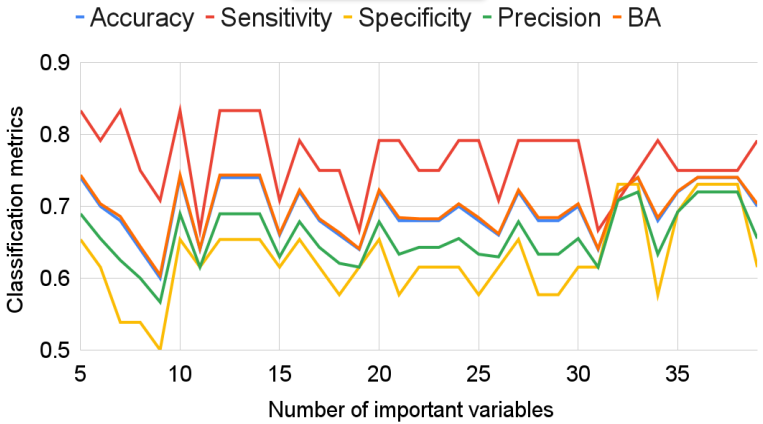
Label 1



ACC=(TP +TN)/(TP+TN+FP+FN)

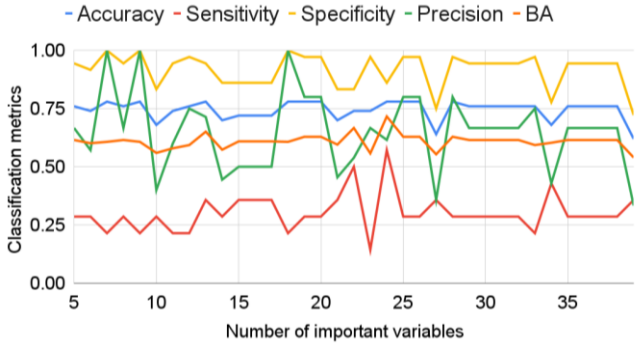
SEN=TP/TP+FN

Label 2



PRE=TP/TP+FP

Label 3



SPE=TN/TN+FP

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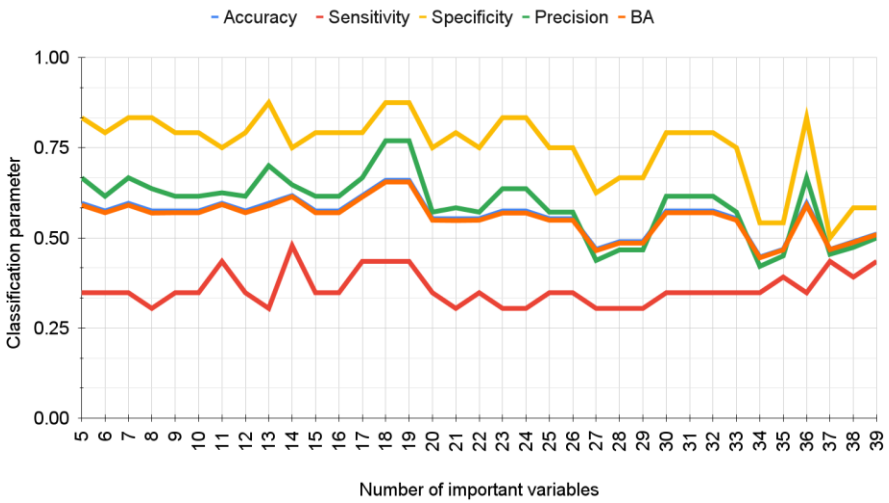
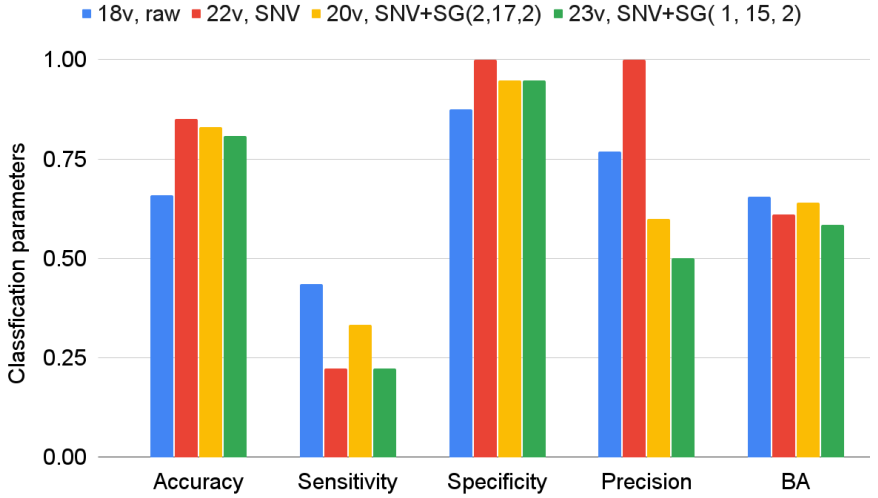
Classification metrics according to different number of important variables as input for PLSDA

Brioso | Raw





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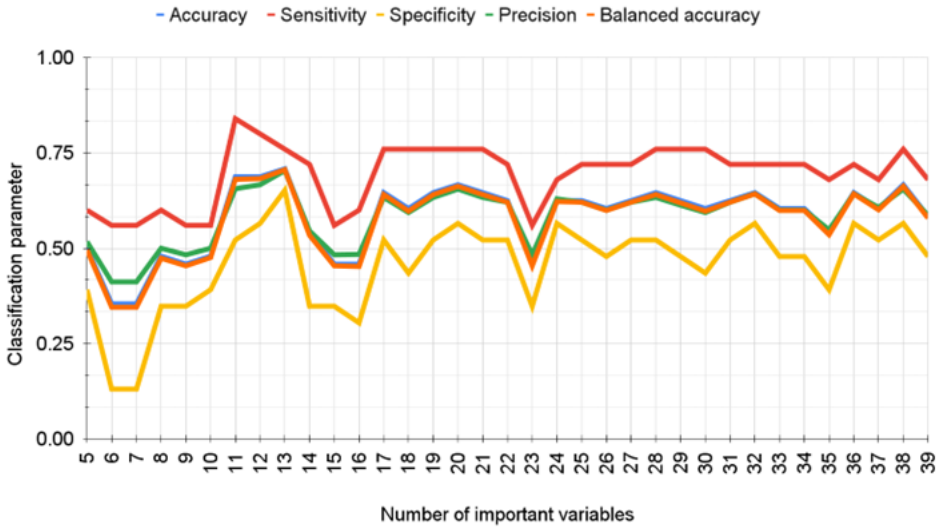
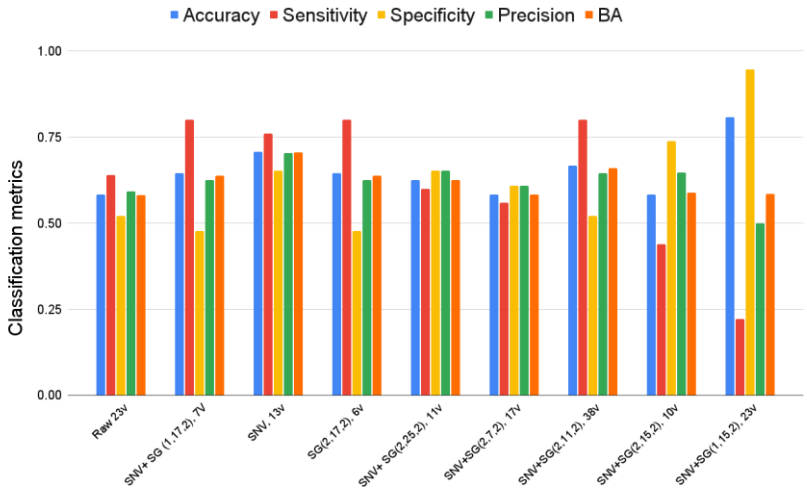
Classification metrics according to different number of important variables as input for PLSDA

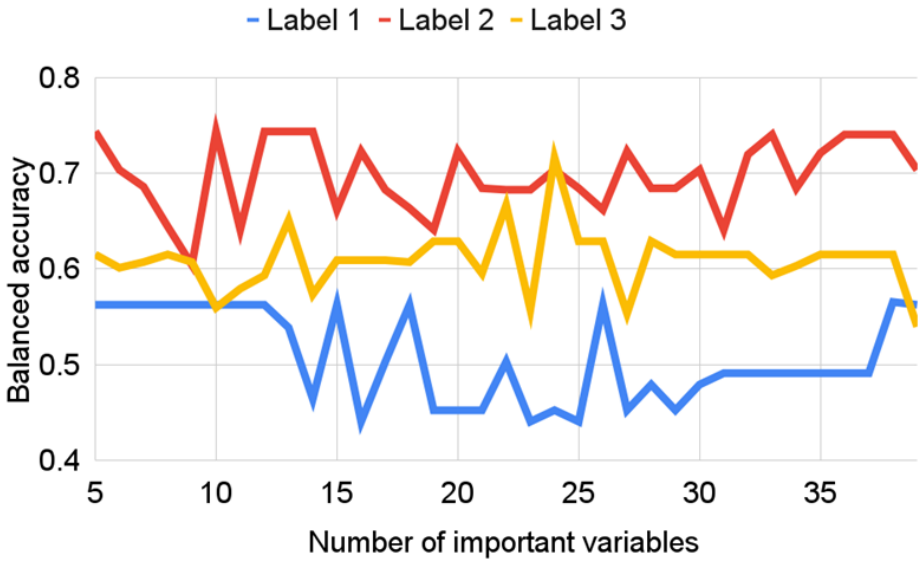
Provine | SNV

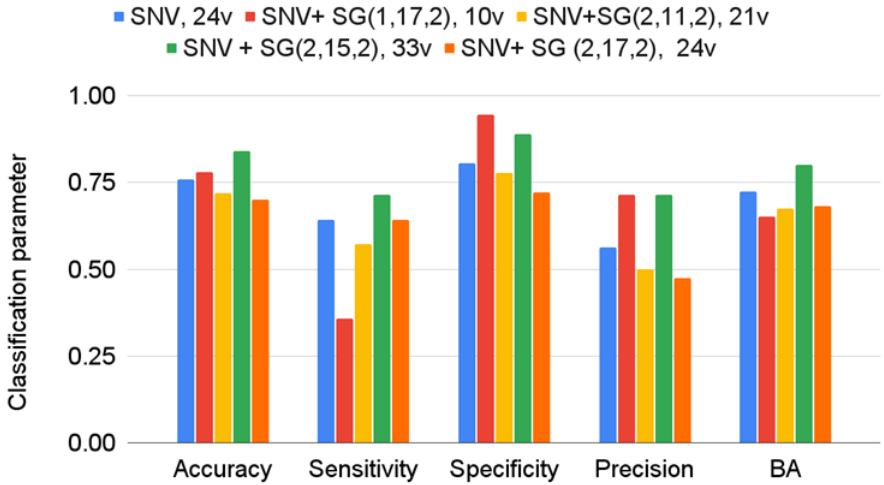




Classification metrics according to different number of important variables as input for PLSDA

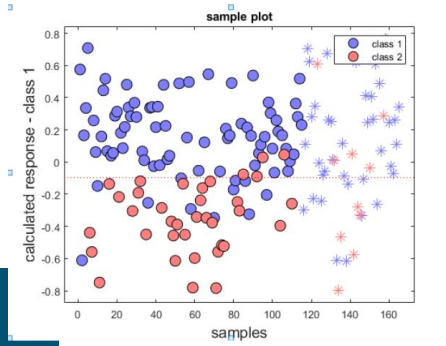
Cappricia | Raw





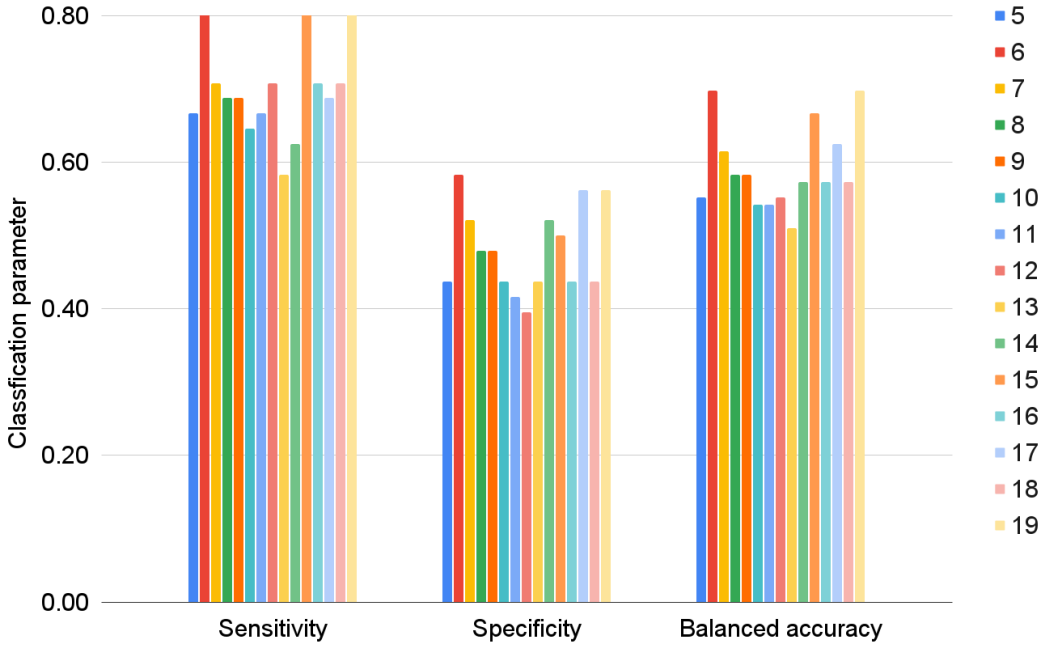
Intervariety Label 2 SNV + SG (2,17,2) LV=3

Cal: Cappricia Val1: Brioso Val2: Provine

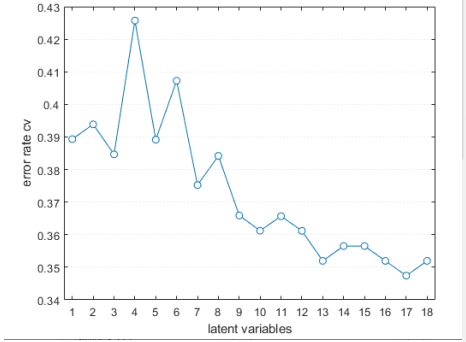


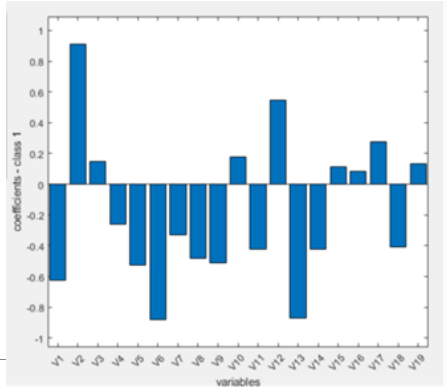
Data set	Real/ predicted	Healthy	Diseased	NA	SEN	SPE	PRE	AC	BA
Calibration Cappriccia	Healthy	66	11	0	0.86	0.55	0.63	0.70	0.71
	Diseased	38	46	0	0.55	0.86	0.81	0.70	0.71
Validation Brioso	Healthy	67	7	0	0.91	0.22	0.54	0.56	0.57
	Diseased	57	16	0	0.22	0.91	0.70	0.56	0.57
Validation Provine	Healthy	80	0	0	1	0.03	0.54	0.54	0.52
	Diseased	69	2	0	0.03	1	1	0.54	0.52

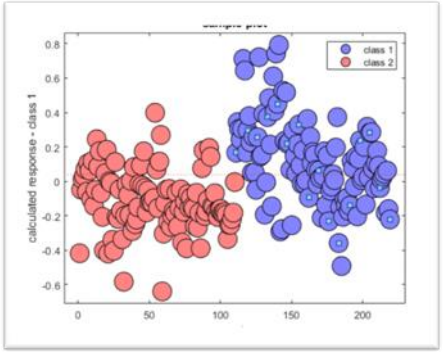
Classification parameters for the global model "Cap&Pro" SNV + SG (2,17,2) according to the number of important variables as input for PLSDA



Global Model





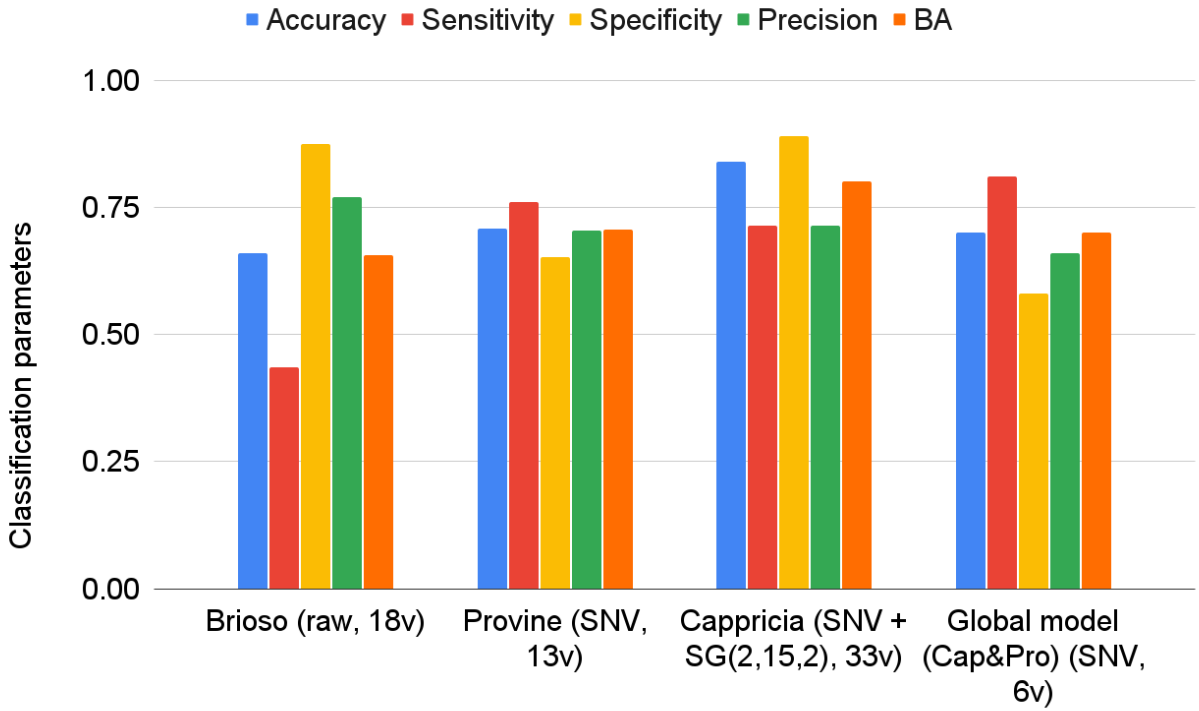


Data set	Real/ predicted	Healthy	Diseased	NA	Sensitivity	Specificity	Precision	BA
Calibration	Healthy	62	47	0	0.57	0.74	0.68	0.66
	Diseased	29	81	0	0.74	0.57	0.63	0.66
Validation	Healthy	23	25	0	0.48	0.71	0.62	0.60
	Diseased	14	34	0	0.71	0.48	0.58	0.60

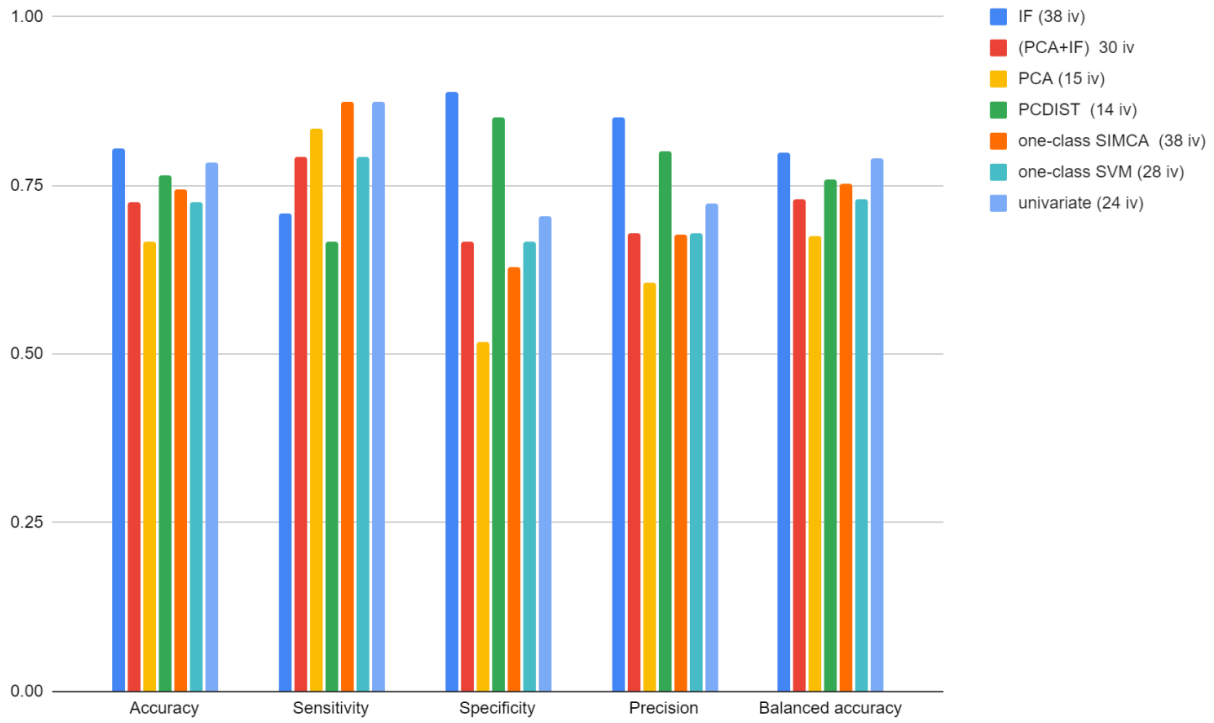
Results- Summary

Parameter/ Model	Cappricia Raw, 15v Label 3	Cappricia SNV + SG(2,15,2), 33v Label 2	Provine Raw, 14v Label 3	Provine SNV, 13v Label 2	Brioso Raw, 18v Label 2	Intervariety Cal: Cap, Val: Bri Raw, 10v Label 3	Global model SNV, (Cap+Pro) 6v Label 2
Accuracy	0.83	0.84	0.71	0.71	0.66	0.61	0.70
Misclassification rate	0.17	0.11	0.29	0.29	0.34	0.39	0.30
Sensitivity or recall	0.89	0.71	0.08	0.76	0.43	0.32	0.81
Specificity	0.64	0.89	0.97	0.65	0.88	0.89	0.58
Precision	0.89	0.71	0.50	0.70	0.77	0.93	0.66
Balanced accuracy	0.77	0.80	0.52	0.71	0.65	0.61	0.70
Geometric mean	0.75	0.79	0.27	0.70	0.62	0.53	0.69
F-measure	0.89	0.71	0.14	0.73	0.55	0.48	0.73
Youden's Index	0.53	0.60	0.05	0.41	0.31	0.21	0.39
Positive likelihood ratio	2.47	6.45	2.67	2.17	3.58	2.91	1.93
Negative likelihood ratio	0.17	0.32	0.95	0.37	0.65	0.76	0.33

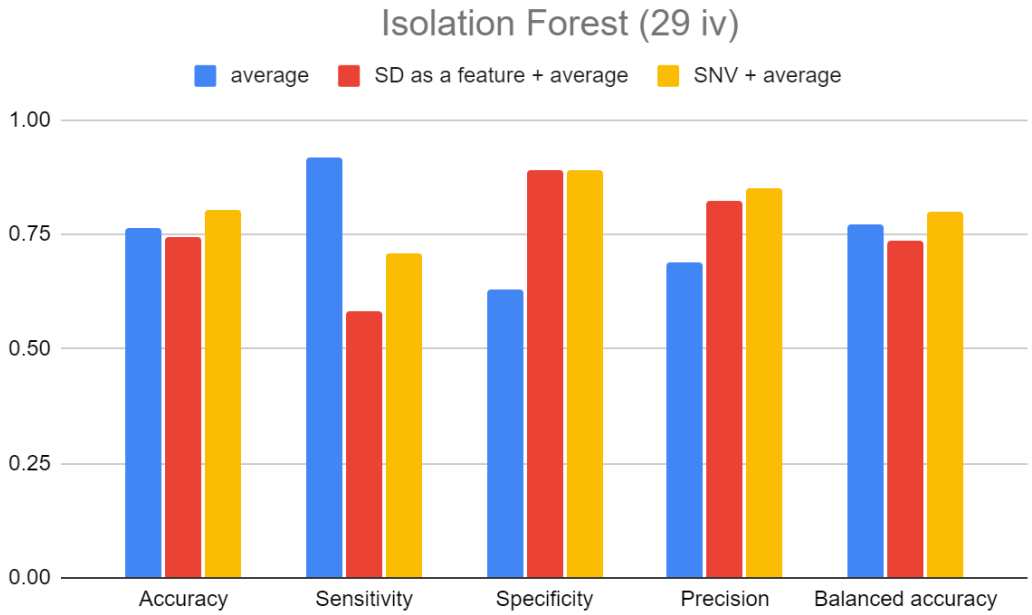
Classification metrics of the optimized models



Comparison of outlier detection methods in terms of their performance in the validation set



The best results in terms of balanced accuracy were found by detecting outliers with Isolation Forest, and performing SNV, then the average of remaining pixels



Results

- Out of all the sepals that did not have the disease, how many got negative test results?

High proportion of actually healthy sepals that were correctly predicted out of all positive predictions made by the models

- Cappricia: 0.71
- Provine: 0.76
- Global model: 0.81

Results

- Out of all the sepals that do have the disease, how many got positive results?

The ability of a test to correctly classify an individual sepal as diseased

- Cappricia: 0.89
- Provine: 0.65
- Global model: 0.58

Results

- A good performance on positive and negative classes respectively was found in Cappricia Intravariety model:

❖ High positive likelihood ratio of 6.45 (above 1: increased evidence for disease-free) for Healthy class

❖ Low negative likelihood ratio of 0.32 (increased evidence for disease) for Diseased class

- BA reduced to the traditional accuracy (0.70) in the global model showing that the classifier performed equally well on either classes

- The intervariety models calibrated in Cappricia showed high sensitivity in class one (0.91) and low specificity in class two; this was consistent in prediction for Brioso (0.03) and Provine (0.11)

Conclusions

Conclusions

- A new global model was calibrated on two different tomato cultivars: Cappricia and Provine; and evaluated in independent samples, using Standard Normal Variate and 6 important variables by CovSel
- The optimized model achieved a sensitivity of 0.81, specificity of 0.58 and balanced accuracy of 0.70
- The model presented potential as a fast alternative method to grade recently harvested tomatoes before the fungal infection is visually observed
- The best results in terms of balanced accuracy were found by detecting outliers with Isolation Forest, and performing SNV, then taking the average of the remaining pixels

Conclusions

- Novelty of this work - investigate HSI to capture the sepal susceptibility of fungal infection by chemometric analysis of different varieties of tomatoes
- The results from this research reaches to a conclusion that discrimination between more susceptible and less susceptible samples is feasible under controlled conditions
- Unanswered questions:
    - What is the information that the spectra captures in these samples to help discriminate the susceptibility to fungus?
    - How will the global model perform when predicting samples from another harvesting time?
- These questions are the likely to set the directions for new investigations on this subject

Thank you for your kind attention

Antares

Horizon 2020
Programme

References

- Brdar, S., Panić, M., Hogeveen-van Echtelt, E. *et al.* Predicting sensitivity of recently harvested tomatoes and tomato sepals to future fungal infections. *Sci Rep* **11**, 23109 (2021). https://doi.org/10.1038/s41598-021-02302-2
- Paper 942-2017 Predictive Accuracy: *A Misleading Performance Measure for Highly Imbalanced Data* Josephine S Akosa, Oklahoma State University https://support.sas.com/resources/papers/proceedings17/0942-2017.pdf
- Roger, J.-M.; Mallet, A.; Marini, F. Preprocessing NIR Spectra for Aquaphotomics. *Molecules* **2022**, *27*, 6795. https://doi.org/10.3390/molecules27206795