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Water Stress Early Detection of Eggplant Plants by Hyperspectral Fluorescence Spectroscopy

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Abstract

Water stress early detection is essential for precision farming to improve crop productivity and product quality. The methods usually used are destructive, long and expensive. In this work, we used hyperspectral chlorophyll fluorescence technology as a rapid, non-destructive approach to detect the water deficiency of eggplant plants using their spectral footprint. So, an experiment was made on 54 eggplant plants subjected to three water treatments: normal irrigation (T_{100}), intermediate irrigation (T_{50}) and no irrigation (T_0). The fluorescence spectra were acquired *in vivo* and *in situ* using a USB4000 spectrometer from Ocean optics. For the classification of the plants subjected to three water treatments, we used three pretreatments of the raw hyperspectral data in order to suppress the non-informative variability present in these spectra and to obtain robust models. These are the Savitzky-Golay smoothing (SG), the standard normal variable (SNV) and the first derivative of Savitzky-Golay (SG-D1). The preprocessed data were then subjected to two partial least squares discriminant analyses (PLS-DA): Hard PLS-DA and Soft PLS-DA. These statistical approaches are suitable for large samples as it reduces the dimensionality of the data but improves the accuracy of the prediction. The SG-D1 combined with the Soft PLS-DA gave the best discrimination of plants with scores of sensitivity, specificity and total efficiency respectively of 97.33%, 94% and 95% for calibration, 6 days after hydric stress induction. For the plants used for the prediction, the scores are 86%, 91% and 90% respectively. This study shows that hyperspectral chlorophyll fluorescence spectroscopy is a fast and non-destructive technology allowing early detection of water stress in eggplant plants.

Keywords

Chlorophyll Fluorescence, Eggplant, Water Stress, Water Deficiency, PLS-DA

1. Introduction

Eggplant is the fruit of a dicotyledonous plant from the Solanaceae family. There are several edible species of cultivated eggplant around the world. The fruits and leaves of all these species are consumed. They have many nutritional qualities. Low in calories, they are rich in fiber, vitamins, antioxidants and minerals beneficial to human health [1] [2] [3]. In Côte d'Ivoire, the cultivation of eggplant occupies an important place in the food crop sector [4] because this plant is consumed in all regions of the country.

This important crop is sensitive to water stress, bacterial, viral, fungal diseases, etc. Because of global climate change, water deficiency is the most damaging abiotic stressor [5]. In agronomy, the water state indicator of plants (implicitly the water stress level) is their water content. In plant physiology methodology, several approaches to determining this parameter are frequently used [6] [7]. However, these methods are destructive, laborious, and lengthy and use few samples [8].

Over the past decade, the technique of hyperspectral chlorophyll fluorescence spectroscopy has evolved rapidly. It is now a new scientific tool for non-destructive assessment of plant stress. Fluorescence emission is directly related to the process of photosynthesis that reflects the physiological state of the plant [9]-[14]. Thus, studying the fluorescence spectrum makes it possible to detect any stress experienced by the plant at the leaf or canopy scale [15]-[21]. Most of these studies use fluorescence ratios at two different wavelengths while for our work, we use the whole spectrum.

The aim of this study is to evaluate the early, rapid and non-destructive detection of water stress in eggplant plants from hyperspectral chlorophyll fluorescence data and to design an appropriate methodology. To achieve this objective, the raw data underwent pretreatment combined with discriminating analysis of partial least squares.

The present work is structured as follows: first, we explain the hydric stress induction and present the experimental setup to acquire the fluorescence spectra. Then, raw data analysis and pretreatment methods in order to discriminate the water-stressed plants are presented. Finally, we compare the results to identify the best method to detect hydric deficiency in eggplant plants.

2. Materials and Methods

2.1. Study Site and Plant Material

The experiment was carried out at Adiopodoumé Km 17 in Côte d'Ivoire at an altitude of 05˚19'27.9''N and a longitude of 04˚08'12.6''W. To effectively control environmental variables, such as temperature, humidity and light, the experiment took place in the greenhouse of the Central Laboratory of Biotechnology of the Centre National de Recherche Agronomique (CNRA). In this greenhouse, the mean temperature and humidity values were 30˚C and 78% respectively. The eggplant variety provided by the CNRA and used in this study is called MEL7TV1.

2.2. Induction of Water Deficit and Experimental Design

The eggplant seeds were sown on a seeding tray. When the seedlings reached the 4 - 5 leaf stage, they were removed from the seeding tray and transplanted into plastic pots. These pots, which had a diameter of 20 cm, a height of 22 cm and a capacity of 5 L, contained a well homogenized soil rich in mineral elements necessary for the good growth of the plant. The bottom of these pots has been pierced to let the water drain after watering to avoid root asphyxiation. Thirty (30) days after planting, the pots were arranged in three random blocks. Each block consisted of 24 plants subjected to three water treatments: normal irrigation (T_{100}), intermediate irrigation (T_{50}) and no irrigation (T_0) with eight (8) plants per treatment. Figure 1 shows the arrangement of plants for each water treatment in the three blocks (B1, B2 and B3).

2.3. Acquisition of Leaf Fluorescence Spectra

As soon as the water deficit was induced, the fluorescence spectra were acquired *in vivo* and *in situ* on 216 leaves, at a rate of 3 leaves per plant. The spectral response of the leaves per plant was obtained from the average of these three measurements. Data collection took place every two days between 07:00 and 11:00 until the first signs of water stress appeared on leaves, 12 days after stress induction (DAI). The data we used for the analysis are all those acquired from 1 DAI to 6 DAI.

The fluorescence spectra acquisition system consisted of a USB 4000 spectrometer, a blue LED excitation source (LS-450), a bifurcated optical fiber and a laptop. Using the blue LS-450 source and the bifurcated optical fiber, the leaf is excited. After excitation, it emits fluorescent light which is sent to the USB4000 spectrometer by the second route of the bifurcated fiber. The fluorescence spectral data stored in the laptop connected to the spectrometer are between 640 and 800 nm with a 0.22 nm sampling pitch. Figure 2 shows the configuration of the hyperspectral fluorescence experimental device.

2.4. Data Analysis

The MATLAB R2018b software was used to analyze hyperspectral fluorescence data. Principal Component Analysis (PCA), Hyperspectral Data Pretreatment Methods and Partial Least Square Discriminant Analysis (PLS-DA) models for water stress early detection in eggplant plants during the asymptomatic period were used.

2.4.1. Principal Component Analysis

Principal Component Analysis is an extremely powerful information synthesis tool when a large quantitative database is available for processing and interpretation. It makes it possible to transform the many highly correlated variables into a reduced number of new uncorrelated variables: these new synthetic variables are called main components [22] [23].

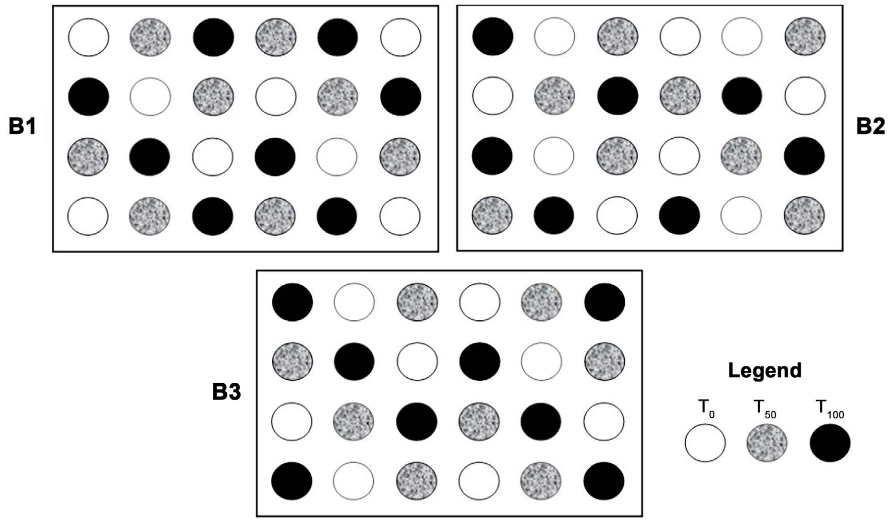

Figure 1. Experimental planting plan for eggplant plants subjected to water stress.

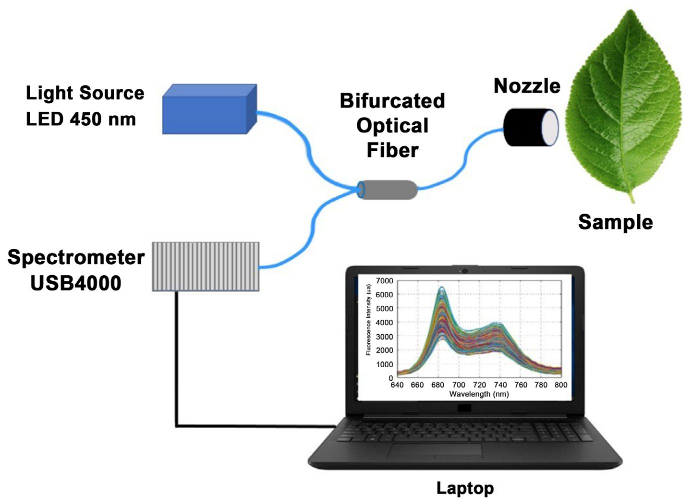

Figure 2. Experimental setup for hyperspectral fluorescence analysis.

In general, the first two main components contain more than 90% [22] [23] of information from the original variables. In addition, the information contained in each variable is not repeated.

In this study, the raw chlorophyll fluorescence spectra of the control plants (T_{100}) and stressed plants (T_{50}, T_0) collected were subjected to main components analysis. Therefore, the first three components were selected based on the cumulative variance rate and used to explore the distinction of plants subject to the three (3) water treatments.

2.4.2. Spectral Pretreatment

After exploration of the raw hyperspectral data, pretreatment was necessary to remove the non-informative variability present in the raw spectra to obtain robust and highly discriminating models. Three pretreatment methods were used to correct the spectral data. These are: Savitzky-Golay (SG) smoothing [24], the standard normal variable (SNV) [25] and the first derivative of Savitzky-Golay (SG-D1) [24].

2.4.3. Partial Discrimination Analysis of Least Squares

Following pretreatment, two multi-class versions of the partial least squares discriminant analysis (Hard PLS-DA and Soft PLS-DA) were used to build models for detecting water stress in eggplant plants.

Partial least squares discriminant analysis is a statistical approach used for large samples as it reduces the dimensionality of the data and maximizes the accuracy of the prediction. This is an appropriate method for highly correlated data. The PLS-DA model was developed using the PLS2 regression constructed between the X and Y matrices where the X matrix is used as a predictor, and the Y matrix with dummy variables represents the response. The regression model is used to compute the predicted responses $\hat{Y}$, which are then used for discrimination. In the traditional implementation of PLS-DA, the discrimination rule is based on the comparison of predicted response values of $\hat{Y}$ with a fixed threshold (e.g. 0.5). In the hard and soft models of PLS-DA, the rule is based on the comparison of a distance between the thousandth line of the $\hat{Y}$ matrix with the corresponding thousandth line of the Y matrix (vector of the pattern response for class *k*). To assess this distance, it was proposed [26] to use the main component analysis of the $\hat{Y}$ matrix, which gives a "super-score" T matrix.

$$X, Y \xrightarrow{PLS2} \hat{\mathrm{Y}} \xrightarrow{PCA} \mathrm{T}$$

The "super-score" T matrix represents a new data set to which a classification method can be applied. We consider two methods: the linear discriminant analysis which provides a hard version of PLS-D and the quadratic discriminant analysis which results in a soft PLS-DA [26].

2.5. Model Evaluation

Our database of 432 spectra was subdivided into 288 spectra for calibration and 144 spectra for prediction. Leave-One-Out cross-validation (LOOCV) was used to determine the main factors of the different models on calibration spectra only. Pomerantsev and Rodionova (2018) [26] proposed three parameters to characterize the overall quality of classification in relation to class k of multi-class models of partial least squares discriminant analysis: total sensitivity (TSE), total specificity (TSP) and total Efficiency (TEF). These are defined by the following equations:

$$TSE = \frac{1}{I}\sum_{k=1}^{K} n_{kk} \tag{1}$$

$$TSP = 1 - \frac{1}{I}\sum_{k \neq 1}^{K} n_{kl} \tag{2}$$

$$TEF = \sqrt{(TSE) \times (TSP)} \tag{3}$$

where n_{kk} represents the number of samples of class k predicted as a member of class k; n_{kl} represents the number of samples of class k predicted as a member of class l and I is the total sample size.

Sensitivity is the ability of the model to correctly identify the class of samples, while specificity is the ability of the model not to be mistaken in the classification. Efficiency represents the correct predictive accuracy of the model.

3. Results and Discussion

3.1. Fluorescence Spectral Signature Analysis

The raw chlorophyll fluorescence spectra of the leaves of all eggplant plants are presented in **Figure 3(a)**. In **Figure 3(b)**, the average spectral profile of the leaves of normal (T_{100}), intermediate (T_{50}) and no irrigation (T_0) plants are plotted in green, blue and red respectively.

The spectral signatures of our samples are similar, regardless of the water state of the plants (**Figure 3(a)**). However, the chlorophyll fluorescence intensities of water deficient plants are lower than those of normal irrigation plants (**Figure 3(b)**). This shows that water stress influences the spectral characteristics of eggplant plants. High chlorophyll fluorescence intensity indicates reduced photosynthetic activity in leaves under water stress due to their low water and chlorophyll contents [10]. The spectral fluorescence responses of eggplant plants obtained are similar to those from other water stress studies conducted on other plants. Our results also show that the fluorescence spectra of eggplant leaves have a chlorophyll *a* fluorescence emission peak in the red at 685 nm and another peak in the near infrared at 735 nm. Various studies on other plant species have shown that these two peaks are between 680 nm and 740 nm regardless of the stress to which they have been subjected [17] [18] [27]. As displayed in **Figure 3(b)**, the spectra of plants with normal irrigation (T_{100}) and those with a water deficiency (T_0 and T_{50}) present large difference. These plants are therefore likely to be discriminated from each other.

3.2. Principal Component Analysis

Figure 4 shows the PCA results obtained using raw spectral fluorescence data from eggplant plants subjected to the three water treatments.

The principal components analysis results show that the first three major components (PC1, PC2 and PC3) express up to 98.24% of the total variance. These principal components have respectively, a variance of 95.94%, 1.64%, and 0.66%. The scatter diagram of the scores (**Figure 4**) for the first three major components of the raw spectra presents good discrimination between T_0 and T_{100}. On the other hand, there is an overlap between T_{50} and the treatments T_{100} and T_0. Although the PCA has reduced the number of spectral data, it is still difficult to effectively distinguish the couples of treatments (T_0, T_{50}) and (T_{100}, T_{50}).

To improve this classification, spectral preprocessing methods will be applied to the raw spectra to establish efficient discrimination models.

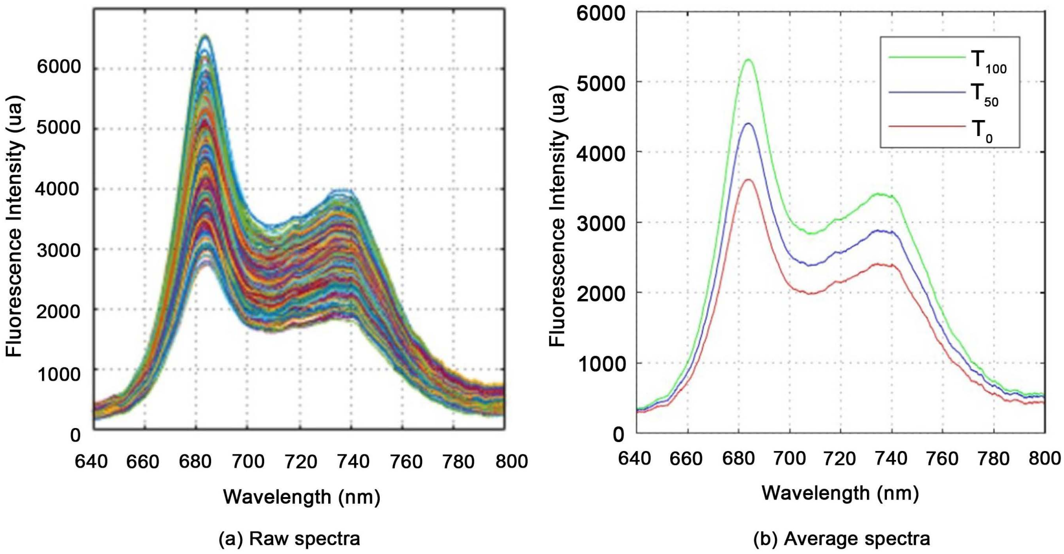

Figure 3. Leaf fluorescence spectra of eggplant plants.

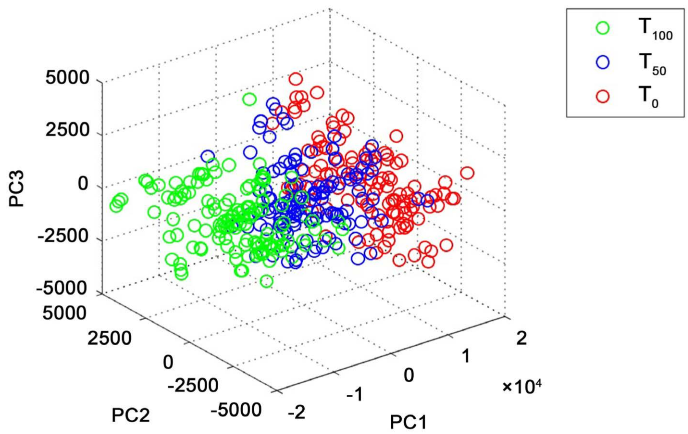

Figure 4. First three main components of the raw spectral fluorescence data of eggplant plants subjected to three water treatments.

3.3. Discriminant Analysis Hard PLS-DA and Soft PLS-DA

The statistics of the water status classification models of eggplant plants, Hard PLS-DA and Soft PLS-DA of the raw and preprocessed spectra, are presented in **Table 1**.

The Hard PLS-DA model obtained a total recognition efficiency of calibration and loss sets greater than 74%. The best model is obtained from raw spectra with a total efficiency of 91% in calibration and 85% in prediction. Applying SG, SNV and SG-D1 preprocessing methods before applying the model does not improve the overall classification efficiency. The total efficiency of SG, SNV and SG-D1 is even lower than that from raw spectra. The results of the best Hard PLS-DA classification model are shown in **Figure 5**.

The Soft PLS-DA model obtained a total recognition efficiency of calibration and loss sets greater than 81%. The SG-D1 preprocessing yielded the best classification model with a total efficiency of 95% in calibration and 90% in prediction,

Table 1. Hard PLS-DA and Soft PLS-DA model results of raw spectra, pre-processed SG, SG-D1 and SNV spectra to identify water status of eggplant plants.

Spectra	Data set	Hard PLS-DA			Soft PLS-DA		
		Total Sensitivity (%)	Total Specificity (%)	Total Efficiency (%)	Total Sensitivity (%)	Total Specificity (%)	Total Sensitivity (%)
RAW	Calibration	91	91	91	97	96	94
	Prediction	85	85	85	81	95	87
SG	Calibration	76	76	76	97	91	94
	Prediction	74	74	74	88	89	88
SG-D1	Calibration	76	76	76	97	94	95
	Prediction	75	75	75	86	91	90
SNV	Calibration	85	85	85	96	82	89
	Prediction	75	75	75	82	80	81

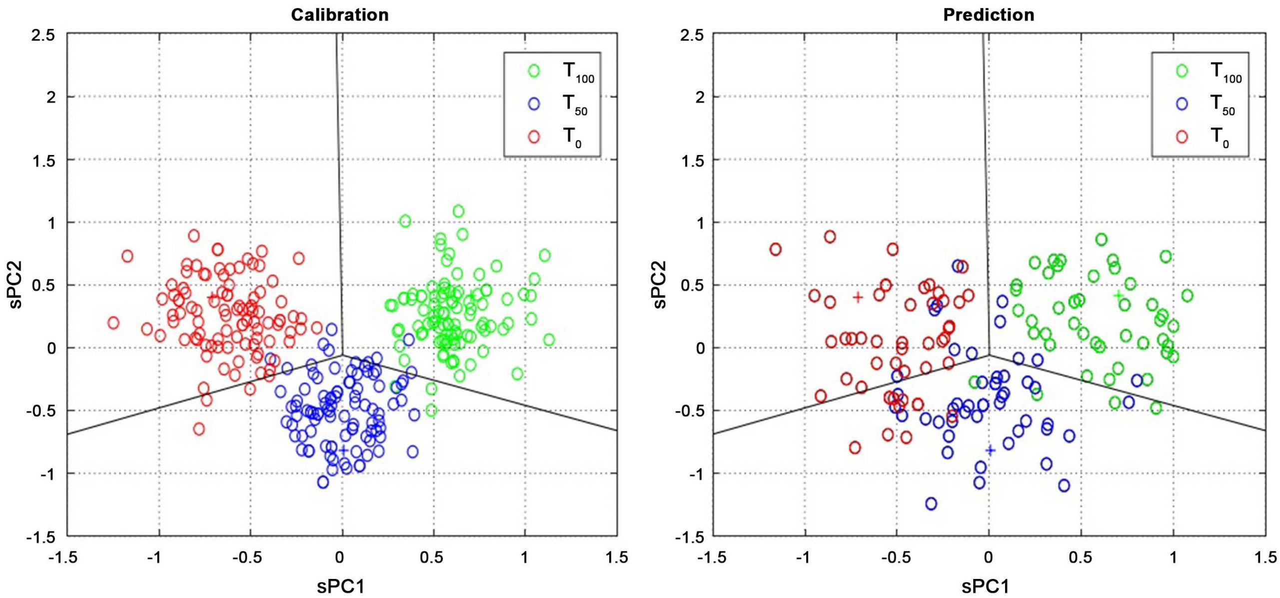

Figure 5. Results of the best classification model of Hard PLS-DA models.

representing a significant improvement in raw data performance. Therefore, Soft PLS-DA model coupled with SG-D1 pretreatment method and raw data could be adopted as an optimal combination to identify water status of eggplant plants. Figure 6 illustrates the results of the best Soft PLS-DA classification model.

Comparing the results of the two multiclass versions of the partial least squares discriminant analysis, the Soft PLS-DA model performed better than the Hard PLS-DA model. The total efficiency of the Soft PLS-DA classification models was found to be higher than that of the Hard PLS-DA classification models, which is consistent with the trends reported by Kunz *et al.* [28] when identifying wood species and by Nunes *et al.* [29] to detect fraud in bovine meat.

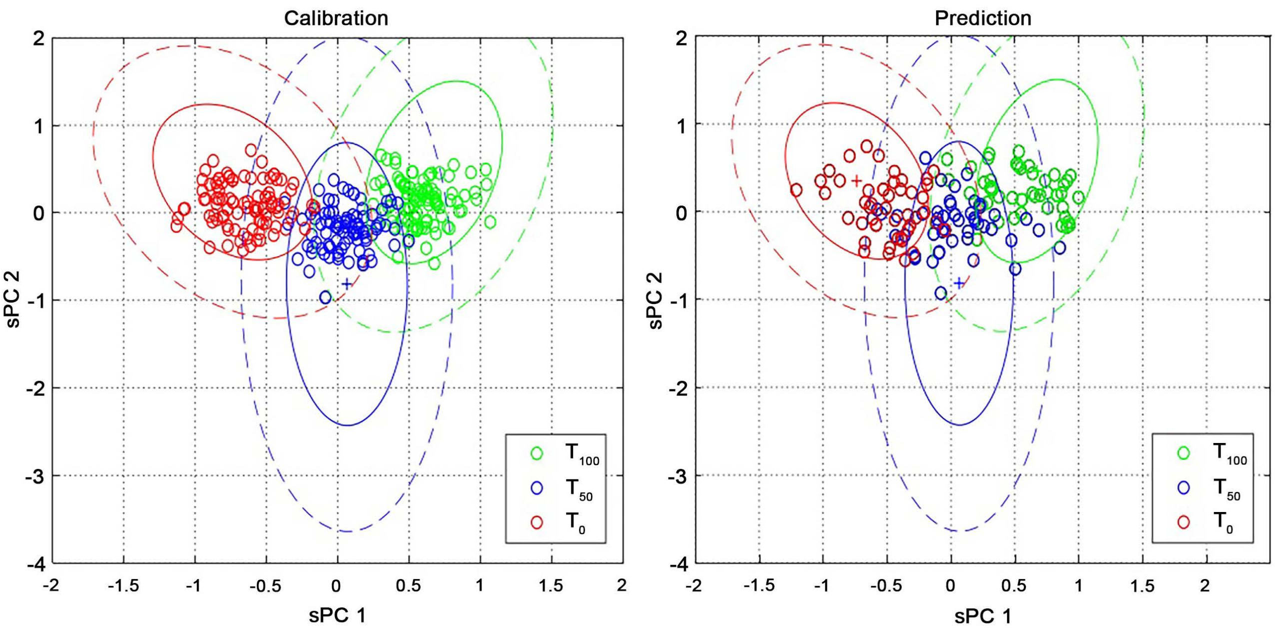

Figure 6. Results of the best classification model of Soft PLS-DA models.

4. Conclusions

Preprocessing methods of hyperspectral chlorophyll fluorescence data from eggplant leaves combined with classification models were applied to build water stress detection models. These models made it possible to detect the water deficiency of eggplant plants six days after stress induction, so before signs of stress are visible on the leaves. The results showed that Savitzky-Golay first derivative combined with soft partial least squares discriminant analysis provided the best discriminant effect, with scores of total sensitivity, total specificity and total efficiency of 97.33%, 94% and 95% respectively for the calibration and 86%, 91% and 90% for the prediction. The control plants (T_{100}) and those not irrigated (T_0) are correctly discriminated. On the other hand, there is an overlap between the pairs of data (T_0, T_{50}) and (T_{50}, T_{100}). However, there is less overlap if the spectral data are subjected to preprocessing.

This study shows that hyperspectral chlorophyll fluorescence spectra can provide early detection of water deficiency in eggplant plants, if these data have undergone preprocessing. This rapid and non-destructive method represents a promising way to monitor the water status of crops during the asymptomatic period.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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