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Non-destructive assessment of chloride in persimmon leaves using a miniature visible near-infrared spectrometer

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Abstract

New methods for the non-destructive, rapid and reliable assessment of leaf chloride (Cl^-) are needed in many agricultural salt-threatened areas throughout the World. Although portable visible near-infrared spectrometers could be useful for this task, accompanying models for leaf Cl^- assessment on basis their visible near-infrared (Vis-NIR) spectra had yet to be developed. To study the feasibility of such models, a persimmon orchard was grown in E Spain and irrigated with eight waters ranging in Cl^- concentration from 70 to 350 mg/L. Then, six times throughout the growing season the Vis-NIR foliar reflectance spectra of various representative leaves per Cl^- treatment were recorded with a portable miniature Vis-NIR spectrometer. The leaf Cl^- was subsequently determined in the same leaves with a laboratory reference method. Next, both the Cl^- and spectral data were analyzed using descriptive statistics, mathematical data transformations, principal components analysis (PCA) and partial least squares regression (PLSR). A wide, sufficiently normal-distributed and representative range of leaf Cl^- content from 0.17 to 3.14 % Cl^- was obtained. With correlation coefficients exceeding +0.65 between 390 and 472 nm and between 690 and 692 nm, the foliar reflectance was the spectral data correlated the most with the leaf Cl^- . Remarkably, both wavelengths intervals are where chlorophyll a has its two absorbance maxima in the visible region. A PLSR model including all the wavelengths in the Vis-NIR reflectance spectra plus the day of the year, attained values of $R^2 = 0.78$, RMSE = 0.34 % Cl^- and RPD = 2.18 in the external validation. However, for leaf Cl^- below 1.5 % Cl^- , i.e., when Cl^- assessment is most needed, this model presented $R^2 = 0.55$, but RMSE = 0.08 % Cl^- and RPD = 5.5, in addition to a bias of +0.04 % Cl^- and a standard error of 0.05 % Cl^- . These results show that portable Vis-NIR spectrometers can be useful for the non-destructive, rapid and even reliable assessment of leaf Cl^- . The potential of this methodology for other crop plants and inorganic ions should be tested.

Keywords: Chloride, Chemometrics, Visible-NIR spectroscopy, Persimmon, Partial least squares

Abbreviations:

<i>A</i> :	Apparent absorbance
$dA/d\lambda$:	First derivative of the apparent absorbance
$d^2A/d\lambda^2$:	Second derivative of the apparent absorbance
Cl ⁻ :	Chloride
Chl <i>a</i> :	Chlorophyll a
Chl <i>b</i> :	Chlorophyll b
DOY:	Day of the year
DW:	Dry weight
K-S:	Kolmogorov-Smirnov
LC:	Latent component
LNC:	Leaf nitrogen content
λ :	wavelength
MLR:	Multiple linear regression
NIR:	Near-infrared
PC:	Principal component
PCA:	Principal components analysis
PCR:	Principal components regression
PLSR:	Partial least squares regression
R ² :	Coefficient of determination
<i>R</i> :	Foliar reflectance
RMSE:	Root mean square error
RPD:	Ratio of standard error of performance to standard deviation
SLSR:	Simple least squares regression
Vis-NIR:	Visible near-infrared

1. Introduction

Chlorine has been long considered as an essential plant nutrient (Broyer et al., 1954). It occurs as chloride (Cl⁻) in plants and, because it readily moves in the sap stream from roots to shoots, but not so much in the other way round, tends to accumulate in leaves and fruits (White and Broadley, 2001; Raveh, 2005; Li et al., 2017). Chloride functions in plants are diverse: counter ion for cations, unspecific osmoticum for turgor maintenance, proton-transferring in photosystem II, stomata opening and closure, etc. (Guskov et al., 2009; Raven, 2017). Chloride contents of 0.02 – 0.2% (DW) are usually enough to fulfil these functions in most plants, i.e., non-halophytes, e.g., all crops (Marschner, 2011). However, under certain conditions crops

accumulate Cl^- in their leaves up to 1.5 – 5% (Xu et al., 2000). Excess leaf Cl^- harms photosynthetic activity in several ways (Geilfus, 2018) and, although plants buffer cytosolic Cl^- concentration by pumping this ion into cell vacuoles through transport proteins (Li et al., 2017; Geilfus, 2018), this process requires energy and thus, inevitably, crop growth and development become impaired. This is an effect of phytotoxicity, which often progresses unnoticed and, when it finally shows up as leaf chlorosis and even necrosis, is much too late to avoid yield losses and other damages (Geilfus, 2018). In many agricultural areas under dry subhumid to arid climates, such as the persimmon plantations in Valencia (E Spain), Cl^- phytotoxicity is an important agronomical problem (Visconti et al., 2017), and early information about leaf Cl^- contents is needed to take measures and reduce harms.

The usual Cl^- assessment in plant leaves is done by means of a destructive laboratory time-consuming wet analysis (Miller, 1998) and, although quicker methods such as those using NIR bench spectrometers in conjunction with partial least squares regression (PLSR) have been developed (de Paz et al., 2016), new faster procedures would be welcome. At this point, portable leaf spectrometers could offer the basis for such methods. Portable leaf spectrometers for agricultural use span from simple devices using very few wavelengths in the visible region to full-range visible near-infrared (Vis-NIR) spectrometers. This kind of equipment is increasingly used for the rapid appraisal of plant nutrient status, mostly for the indirect estimation of leaf nitrogen content (LNC) through the measurement of leaf transmittance, reflectance and/or fluorescence properties (Padilla et al., 2018; Ali et al., 2017), however, their ability for other element estimations is yet to be explored.

Elements such as chlorine, which are mostly present as free hydrated ions in plants, are by themselves spectroscopically inactive in the whole Vis-NIR range (Shao et al., 2012). Therefore, their detection and eventual quantification using Vis-NIR radiation, has to be done taking advantage of their biochemical and physicochemical effects on the spectroscopically-active compounds present in plants. These can be either pigments, such as chlorophylls, carotenoids and flavonoids, in the case of the visible radiation and, additionally, many other colorless organic compounds in the case of the near-infrared (van Maarschalkerweerd et al., 2013). In the NIR region, hydrogen bonding of Cl^- to peptide bonds in proteins ($>\text{N}-\text{H}\cdots\text{Cl}^-$) was identified as the foundation for Cl^- assessment in dry leaves with excellent coefficients of determination (de Paz et al., 2016). In fresh leaves, however, the plentiful of water molecules are expected to get Cl^- away from most peptide groups in proteins and, therefore, the ability of NIR spectroscopy for Cl^- assessment in this material is deduced to be much weaker. Regarding the leaf water content, its interference is general in LNC estimation with Vis-NIR spectrometers (Samborski et al., 2009). On the contrary, with regard to visible radiation, it has been suggested

that Cl^- could facilitate chlorophyll degradation (Geilfus, 2018), thus providing an important biochemical effect for revealing itself in the leaf visible spectrum.

In order to use all the possible effects of Cl^- on the Vis-NIR leaf spectrum for its assessment without sample destruction, chemometric techniques such as PLSR are the most promising. This is due to the ability of PLSR to optimally take advantage of the whole available spectral information in the range of interest without being hampered neither by the high number of predictive variables, i.e. the wavelengths, nor by the collinearities amongst them. Based on all the predictive variables, PLSR builds several linear functions, or latent components (LCs), that are uncorrelated amongst them and, simultaneously maximize covariance with the analyte of interest (Abdi, 2007), thus overcoming the limitations of multiple linear regression (MLR) models (Tobias, 2003). Furthermore, the performance of PLSR models can be improved by means of data transformations such as derivation, mean-centering and others. All of them increase the signal-to-noise ratio, thus revealing the minute effects on spectra of otherwise difficult-to-detect analytes (Zhang et al., 2019).

Therefore, the objective of this investigation was to study the ability of a portable miniature visible near-infrared spectrometer for the non-destructive assessment of Cl^- in plant leaves. Specifically, this study was performed on persimmon (*Diospyros kaki* L.) using PLSR.

2. Materials and methods

2.1. Experimental persimmon orchard

In 2016 a persimmon orchard was established in the IVIA Agricultural Experimental Station in Moncada (València, Spain) by planting in soil of 48 five-year-old trees of *Diospyros kaki* grafted on *Diospyros lotus* scions. In order to have both the development and wide range of leaf Cl^- contents reported for the commercial persimmon plantations in the Valencia cropping area, the 48 trees were, on the one hand, equally fertilized, and, on the other hand, exposed to different Cl^- water contents by splitting them into eight groups or Cl^- treatments. Therefore, during the growth seasons from April to October of 2016 and 2017, each treatment was irrigated with a different synthetic solution made by dissolving in desalinated water of the same amounts of mineral fertilizers (KNO_3 , $\text{Ca}(\text{NO}_3)_2$, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, H_3PO_4 75%, Fe-EDDHA, H_3BO_3 , ZnSO_4 , MnSO_4 , and H_2MoO_4) and increasing amounts of NaCl and CaCl_2 so as to obtain 8 Cl^- water concentrations between 70 and 350 mg/L separated by a constant increment of 40 mg/L.

2.2. Leaf sampling

Six times throughout the growing season of 2017 several leaves from each treatment were cut and joined into composite samples. The cuttings were done in May 4th, June 1st, June 27th, August 2nd, September 12th and October 30th. In the first five samplings 20 leaves per treatment were collected, whereas in the last sampling 20 leaves were also collected, but from each tree instead. This way we tried to increase the number of samples high in Cl⁻ since we knew that Cl⁻ accumulates throughout the growing season (Visconti et al., 2017). Therefore, we had 8 treatments $\times$ (5 samplings $\times$ 1 sample per treatment + 1 sampling $\times$ 1 sample per tree $\times$ 6 trees per treatment) - 5 lost samples = 83 leaf samples. These lost leaf samples were from trees severely damaged. The leaves were randomly selected amongst those fully extended, usually the third one from the tip of as many different shoots as possible. Once collected, the leaves were immediately sealed in plastic bags and carried to the laboratory at air temperature since the experimental field was just 250 m distant.

2.3. Spectral measurements of the leaves

Once in the laboratory the foliar reflectance (*R*) from the upper side of the leaves between 345 and 1040 nm was recorded. In the first five samplings six leaves randomly selected from each treatment were measured, whereas in the last sampling six leaves also randomly chosen, but this time from each tree, were measured. A place to the left of the leaf midrib between the second and third vein was selected for measurement.

The CI-710 Miniature Leaf Spectrometer (CID Bio-Science, Inc., Camas, Washington, USA) was used to record the *R* spectra. This fully portable spectrometer is able to measure the Vis-NIR transmittance, absorbance or reflectance spectrum between 345 and 1050 nm of a leaf area just 6 mm². Its radiation source combines a blue LED and an incandescent lamp and a clip system protects the measurement area from the interference of the environmental light. Adjusting the measurement specifications of i) number of average scans per recorded spectrum, ii) integration time and iii) boxcar width, the user can smooth the spectra and maximize the signal-to-noise ratio (CID Bio-Science, 2013). For these parameters several values were tried in the first leaves, and the most adequate were found to be, respectively, 4, 400 ms and 10. Then, these values were systematically used for all the *R* measurements.

2.4. Reference leaf chloride contents

Following the *R* measurements all the leaves from the same treatment (first five samplings) or the same tree (last sampling) were gathered for Cl⁻ determination following the reference method of Miller (1998). The sample pretreatment included washing with a nonionic detergent solution and then rinsing with deionised water. Next, the leaves were frozen into liquid nitrogen,

freeze-dried in a Lyoalfa (Telstar, Barcelona, Spain) freeze-drier and grounded in a water refrigerated mill (IKA M 20, IKA Labortechnik, Staufen, Germany). Next, half a gram of the dry and grounded leaf material was shaken in 40 mL of 2% acetic acid for 24 h (Gilliam, 1971), and the Cl⁻ concentration in this extract was determined by argentometry using a coulometric-amperometric titration (Cotlove, 1963) by means of the Chloride Analyser 926 (Corning Ltd., Halstead, Essex, UK). Finally, the Cl⁻ contents were expressed on a dry weight base.

2.5. Processing of the spectral data of the leaves

The foliar reflectance (R) spectra were summed up to have 251 equally spaced data per spectrum within a narrower interval from 380 to 1000 nm. This was done by linear interpolation of the reflectance value at the new wavelengths of 380 nm, 382.48 nm, 384.96 nm, etc., until 1000 nm, using the two immediate lower and higher old wavelengths to each of these new ones. Then, the R spectra from the same treatment (first five samplings) or the same tree (last sampling) were averaged to have, respectively, one mean spectrum per treatment or tree. In the following step, the absorbance (A) was calculated from the foliar reflectance according to the formula $A = -\log R$, and then, the first and second derivatives of the absorbance were calculated using equations 1 and 2:

$$\left. \frac{dA}{d\lambda} \right|_{(\lambda_{m+1}-\lambda_m)/2} = \frac{A_{m+1} - A_m}{\lambda_{m+1} - \lambda_m} \quad [1] \quad \left. \frac{d^2A}{d\lambda^2} \right|_{(\lambda_{m+1}-\lambda_m)/2} = \frac{\left. \frac{dA}{d\lambda} \right|_{m+1} - \left. \frac{dA}{d\lambda} \right|_m}{\lambda_{m+1} - \lambda_m} \quad [2],$$

where λ is the wavelength, and m indicates the order from the first to the last wavelength.

2.6. Exploratory data analyses

Several exploratory data analyses were carried out with the aid of the R packages ‘stats’, ‘moments’ and ‘nortest’ which were built under R software versions, respectively, 3.5.1, 3.5.2 and 3.5.2 (R Core Team, 2018). First of all, the distribution of the Cl⁻ contents in the leaves was studied by assessing skewness with the Pearson’s moment coefficient, and normality with the Kolmogorov-Smirnov and Lilliefors tests. Additionally, the Pearson’s product-moment correlation coefficients between Cl⁻ contents and the spectral datasets, throughout the interval from 380 to 1000 nm were calculated. Finally, the principal components analyses (PCAs) of the matrix of correlation coefficients between, respectively, R , A , $dA/d\lambda$ and $d^2A/d\lambda^2$ at each wavelength were done. The maximum number of PCs with eigenvalue over 1 was taken into account for the ensuing model developments with PLSR.

2.7. Model developments with SLSR and PLSR

The 83 rows of Cl^- content plus spectral data were split into one training set made up of $\frac{3}{4}$ of the rows, i.e. 62 rows, and one testing set made up of $\frac{1}{4}$ of the rows, i.e. 21 rows. The splitting was done by systematically assigning the $(4i + 3)^{\text{th}}$ row of data, where i is equal to 0, 1, 2, etc., to the testing set and the remaining rows to the training set. The training set was used for the model calibrations and leave-one-out cross-validations. First of all, simple least squares regression using the wavelength of maximum correlation with leaf Cl^- content was applied to obtain a basic benchmark model for subsequent comparisons. Next, the spectral data were mean centered given mean-centering improves modeling because it helps to reduce multicollinearity between predictive variables (Marquardt, 1980). Then, partial least squares regression (PLSR) was used to develop various predictive models differing in their number of LCs. The calibrations and cross-validations of PLSR models were done with the software ParLeS (Viscarra Rossel, 2008).

2.8. External validation of the PLSR model

The selected PLSR models were additionally subjected to an external validation through prediction of the Cl^- contents in the leaves of the testing set and comparison with the reference contents.

3. Results and discussion

3.1. Chloride contents in the leaves

The leaf Cl^- increased throughout time during the growing season, i.e. with the day of the year (DOY), in all treatments (Fig. 1), and from a minimum of 0.17 up to a maximum of 3.14 % Cl^- (Table 1). These extreme values in addition to a mean of 1.30 % Cl^- compare well to the leaf Cl^- contents found in many commercial persimmon plantations in the Valencia cropping area. In the training set the Cl^- contents were between 0.17 and 3.14% with mean of 1.29%, while in the testing set were between 0.22 and 3.05% with mean of 1.33%.

[Figure 1]

Concerning the leaf Cl^- statistical distribution, it exhibited a positively skewed one that, however, did not significantly depart from normality according to the Kolmogorov-Smirnov test, and not even with the more demanding Lilliefors test (Table 1). As a consequence, although somewhat skewed, the Cl^- data could be regarded as to come from a normal-distributed population.

[Table 1]

3.2. Visible Near-infrared spectra

The Vis-NIR foliar reflectance and absorbance spectra of all leaf samples along with the first and second derivatives of the absorbance are shown in Fig. 2.

[Figure 2]

The correlation coefficients (r) between leaf Cl^- and foliar reflectance were between 0.05 and 0.77 with up to 37 wavelengths remarkably correlated, i.e., with r in absolute value exceeding 0.65 (Fig. 3 and Table 2). The correlation coefficients with absorbance reversed, however. Besides, both the maximum in absolute value and the number of remarkably correlated wavelengths decreased to, respectively, 0.66 and 11. First derivation recovered the maximum correlation with Cl^- content but lost wavelengths with $|r|$ over 0.65, whereas an additional derivation worsened correlations even more.

[Figure 3]

[Table 2]

The maximum correlation in absolute value of foliar reflectance with Cl^- content occurred at 412 nm. The other wavelengths with remarkable correlation coefficients in absolute value were all in the visible region: between 390 and 472, 626, 643 and between 690 and 692 nm, whereas in the NIR, i.e., between 700 and 1000 nm, none appeared. The leaf chlorophylls, i.e., Chl a and Chl b , and additionally, other important pigments such as β -carotene, absorb light in the visible range where they present several absorbance maxima, remarkably between 400 and 500 nm and between 650 and 700 nm (Fig. 4). Since the maximum correlation of leaf Cl^- as shown in Fig. 3 matches the two wavelengths intervals of maximum absorbance by Chl a (Fig. 4), it seems that Cl^- interacts specifically with Chl a to show up in the leaf spectra. Moreover, given that its correlation with foliar reflectance is positive, then Cl^- acts by decreasing either Chl a content or its light absorption. Romero et al. (1997) found that the contents of chlorophylls in melon leaves decreased as a consequence of Cl^- , thus the same biochemical degradative effect likely occurs in our persimmon leaves.

[Figure 4]

These results obtained for the Vis-NIR region up to 1000 nm complement those attained by de Paz et al. (2016), who found that Cl^- interaction with peptide bonds changes the leaf NIR spectrum between 1200 and 2400 nm. In the NIR region between 1200 and 2400 nm, however, the effects of Cl^- were only clearly seen in the first derivative of the absorbance, thus they changed the steepness of the absorbance maxima and minima there. Conversely, in the case of the Vis-NIR region between 380 and 1000 nm analyzed in this work, the interaction of Cl^- with

the leaf organic compounds mainly changes the magnitude of their absorbance and, hence, the foliar reflectance. Therefore, the Cl^- effects in the leaf visible spectrum are stronger than in the NIR one and thus, foliar reflectance itself is the most adequate property for Cl^- prediction using Vis-NIR spectroscopy. The foliar reflectance was thus selected for modeling.

3.3. Principal components analyses

In the foliar reflectance spectrum up to 6 principal components (PCs) had eigenvalues exceeding one, and these jointly accounted for 99.5% of variance. The DOY was included along with the foliar reflectance spectrum, however, this barely changed the amount of variance explained by the six first PCs, and it neither changed the number of PCs with eigenvalue over unity. This high amount of variance explained by so few PCs reveals, firstly, that there are important collinearities amongst all the wavelengths in the foliar reflectance and, secondly, that DOY is also highly correlated with all these wavelengths. In spite of this high correlation, DOY could still provide important predictive information because of the evolution of foliar reflectance throughout the growing season, which is what DOY measures and thus, DOY could be profitably added into a PLSR model.

3.4. Partial least squares regressions

In the first place and for the sake of parsimony, a simple least squares regression (SLSR) was carried out using the foliar reflectance wavelength in the training set that featured the maximum correlation with leaf Cl^- . This was 412 nm (Figure 3 and Table 2). According to the leave-one-out cross-validation used to test this SLSR model, the following values for the fit parameters were obtained: $R^2 = 0.548$, $\text{RMSE} = 0.541 \text{ \%Cl}^-$ and $\text{RPD} = 1.498$. This model could be improved by including more variables, such as other wavelengths and even DOY following an MLR approach. However, as the previous PCA revealed, all the wavelengths in the reflectance spectrum are highly correlated amongst them and, additionally, with DOY. This fact would lead to an MLR model with regression coefficients severely inflated in variance, i.e., unstable and, definitely, to a potentially misleading model (Gunst, 1983). In such a case of multicollinearity the best alternative for model development is PCR or PLSR. However, given that PLSR is particularly adequate when, in addition to multicollinearity, there are more predictive variables than cases (Wold et al., 2001; Abdi, 2007), as happens in this investigation, PLSR was selected.

Since 6 was the number of PCs with eigenvalues over one, PLSR was applied to develop up to $6 \times 2 = 12$ models by using, on the one hand, all the wavelengths with foliar reflectance data and, on the other hand, all these plus DOY. In Table 3, the fit parameters R^2 , RMSE and RPD of

various models with between 6 and 12 LCs are shown. As the number of LCs increased, the R^2 also increased until 9 LCs without DOY and until 8 LCs with DOY. The assessment of RPD complements the interpretation of R^2 , and it is shown that the RPD kept under 1.75 for models without DOY and over 1.75 for models using DOY. Again, the RMSE only presented values below 0.47 %CI⁻ for the models using DOY. On average the R^2 , RMSE and RPD were, respectively, 0.05 higher, 0.04 %CI⁻ lower and 0.15 higher for the models using DOY. The fit between predictions and observations in the training dataset can be visually appreciated to the left of Fig. 5.

[Figure 6]

The enhanced fit between leaf CI⁻ predictions and observations caused by the inclusion of DOY in the PLSR model development confirms that, on its own, elapsed time throughout the cropping season influences leaf spectra. Therefore, the inclusion of DOY in the model would take into account the variation that time throughout the season contributes to reflectance and, thus, would contribute to isolate the effect of just CI⁻.

The DOY was, therefore, included in the model. In Fig. 6 it is graphically explained how DOY influences the foliar reflectance through two different effects: one direct and another indirect by means of CI⁻. On the one hand, the leaf chlorophyll contents change throughout the growing season, i.e. as DOY increases (Hörtensteiner and Kräutler, 2011). On the other hand, in the case of trees subjected to CI⁻, the leaf content of this anion also increases throughout the growing season, i.e. as DOY increases, as it occurred in our persimmon orchard (Fig. 1). Since excess CI⁻ likely eases chlorophyll, specifically Chl *a*, degradation, leaf reflectance also indirectly changes, particularly decreases, with time as a consequence of CI⁻ increase.

[Table 3]

[Figure 5]

3.5. External validation of the PLSR models

All the models built in the previous calibration were used in the testing dataset and observations were compared with predictions by means of the fit parameters R^2 , RMSE and RPD (Table 3) in addition to a graph (Fig. 5 right). The R^2 reached values over 0.75 for several models either using or not using DOY. Besides, RPD overcame 2 and RMSE dropped below 0.4 for almost the same models. Taking into account both the cross- and the external validations, the model using DOY plus all the wavelengths in the foliar reflectance spectra and, then, 8 LCs was the selected one. This model has been highlighted in italics in Table 3, and the fit between model

predictions and reference leaf Cl^- contents in the training and testing datasets can be visually appreciated in Fig 4. For the benefit of the reader, the regression coefficients, loading weights and spectral LC loadings of this model have been provided as Electronic Supplementary Material (ESM) in the form of a spreadsheet. According to the plots of residuals in the training dataset, the errors of the selected PLSR model can be regarded as normally-distributed, however, they slightly increase with Cl^- content (Fig. 7).

[Figure 7]

The RPD values featuring the selected model both in calibration and validation were well below 3 indicating that the selected model could be regarded as acceptable for estimation but not for determination purposes (Fearn, 2002). This RPD values contrast with the ones obtained for leaf Cl^- assessment using dry and grounded leaves and the NIR absorbance first derivative between 1200 and 2400 nm (de Paz et al., 2016), which in that instance were over 4.5 in cross-validation and 3.5 in external validation for the whole 0 – 3 % Cl^- range. In the present research, nevertheless, two zones separated by 1.5 % Cl^- can still be distinguished in the residuals plot (Fig. 7) and, the lower one better adapted for prediction purposes. In this area of less than 1.5 % Cl^- , RMSE drops to 0.08 % Cl^- and RPD reaches 5.5. It is interesting to note that for Cl^- contents over 1.3 % Cl^- , leaf burn inevitably shows up in persimmons, i.e., it becomes visible (Visconti et al., 2017). It is, therefore, for low leaf Cl^- contents (< 1.3 % Cl^-) for which predictions are most needed. Given that below 1.3 % Cl^- is when the selected model better fulfils the requirements for prediction, it is worth using at least there. In this zone of low Cl^- content, the method standard error is 0.05 % Cl^- . This is seven times more than the reference method and almost double than the laboratory method based on NIR that was developed by de Paz et al. (2016). Besides, the method presented here has a bias of just +0.04 % Cl^- , i.e., it barely overestimates Cl^- content on average.

4. Conclusions

The Cl^- contents in persimmon leaves were found to be remarkably and positively correlated with the foliar reflectance between 390 and 472 and between 690 and 692 nm. Both wavelength intervals correspond to the two absorption maxima Chl *a* has in the visible region. These high correlations point to the fact that Cl^- interacts with this key leaf pigment, maybe easing its degradation, and thus making it possible to use Vis-NIR spectroscopy for the assessment of this otherwise Vis-NIR inactive anion. Notwithstanding this, since leaf chlorophylls also naturally evolve throughout the growing season, an additional time variable, i.e., DOY, had to be included in order to refine a predictive model for leaf Cl^- . According to the cross-validation and subsequent external validation, the adequate number of latent factors to be included in such a

model based on PLSR, was 8. This model had a R^2 of 0.78, a RMSE of 0.34 %Cl⁻ and a RPD of 2.18. Besides, although for leaf Cl⁻ contents below 1.5 %Cl⁻ this model presents R^2 of 0.55, its performance improves in terms of RMSE (0.08 %Cl⁻), and RPD (5.5). Additionally, with bias of +0.04 %Cl⁻ and standard error of 0.05 %Cl⁻ this PLSR model is barely affected by systematic errors, though has a standard error seven times the reference laboratory wet method. This latter fact increases the relative standard deviation of this leaf Cl⁻ assessment method to 20%. In conclusion, the use of portable Vis-NIR spectrometers in conjunction with PLSR models can be recommended for the non-destructive, rapid and, at least under 1.5 %Cl⁻, even reliable assessment of leaf Cl⁻ in persimmon orchards. The potential of this methodology for other crop plants and inorganic ions should be tested.

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Declarations of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Figure captions

Figure 1. Chloride contents determined in the persimmon leaves throughout time during the growing season (DOY)

Figure 2. Foliar reflectance (R), absorbance (A) and first ($dA/d\lambda$) and second ($d^2A/d\lambda^2$) derivatives of the absorbance

Figure 3. Pearson's product moment correlation coefficients between the reference leaf chloride and the foliar reflectance (R), absorbance (A), first derivative of the apparent absorbance ($dA/d\lambda$), and second derivative of the apparent absorbance ($d^2A/d\lambda^2$)

Figure 4. Absorbance spectra of the main pigments in the leaves of crop trees (Source: own preparation using absorption coefficients from Taniguchi et al. (2018) and taking the following percents of chlorophyll a, chlorophyll b and β -carotene: 62.4, 19.3 and 18.4%)

Figure 5. PLSR-predicted against observed (reference) leaf chloride in the training and testing sets

Figure 6. Two-way influence of time throughout the growing season, i.e. day of the year (DOY) on the leaf spectrum

Figure 7. Plot of residuals against predicted chloride content (left) in the testing dataset, and plot of expected against observed (reference) cumulative probabilities of chloride content also in the testing dataset (right)

Table captions

Table 1. Statistical summary of the chloride contents in the persimmon leaves

Table 2. Summary of the Pearson’s product-moment correlation coefficients (r) between the leaf chloride content and the four spectral datasets

Table 3. Results of the leave-one-out cross-validations and external validations of the PLSR models to predict leaf chloride on base the foliar reflectance, either with or without DOY, and with different numbers of latent components (LCs). In italics the selected model

Figure 1.

531 **Figure 2.**

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534 **Figure 3.**

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537 **Figure 4.**

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Figure 5.

Figure 6.

Figure 7.

600 **Table 1.**

Descriptor	Complete set	Training set	Testing set
N	83	62	21
Mean (%CI)	1.304	1.295	1.332
SD (%CI)	0.791	0.810	0.749
Maximum (%CI)	3.144	3.144	3.054
Minimum (%CI)	0.166	0.166	0.22
Skewness coefficient	0.397	0.429	0.292
K-S & Lilliefors's statistic	0.078	0.100	0.092
K-S's p-value	0.695	0.533	0.987
Lilliefors's p-value	0.245	0.129	0.914

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620 **Table 2.**

Spectral dataset	Minimum	Maximum	Maximum absolute value	Number of wavelengths with $ r \geq 0.65$
Reflectance (R)	0.054	0.772	0.772	37
Absorbance (A)	-0.664	-0.100	0.664	11
Absorbance 1 st der. ($dA/d\lambda$)	-0.742	0.650	0.742	4
Absorbance 2 nd der. ($d^2A/d\lambda^2$)	-0.651	0.620	0.651	1

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Table 3.

DOY	Number of LCs	Cross-validation			External validation		
		R ²	RMSE (%CI)	RPD	R ²	RMSE (%CI)	RPD
No	6	0.599	0.51	1.58	0.679	0.42	1.80
No	7	0.623	0.50	1.63	0.694	0.41	1.84
No	8	0.642	0.49	1.66	0.792	0.34	2.20
No	9	0.676	0.46	1.75	0.745	0.38	1.99
No	10	0.672	0.47	1.73	0.738	0.38	1.95
No	11	0.672	0.47	1.73	0.699	0.42	1.79
No	12	0.667	0.47	1.71	0.653	0.46	1.64
Yes	6	0.671	0.46	1.75	0.707	0.40	1.85
Yes	7	0.687	0.45	1.79	0.751	0.37	2.03
Yes	8	0.724	0.42	1.91	0.782	0.34	2.18
Yes	9	0.707	0.44	1.84	0.798	0.33	2.25
Yes	10	0.718	0.43	1.88	0.768	0.35	2.11
Yes	11	0.713	0.44	1.85	0.731	0.38	1.95
Yes	12	0.699	0.45	1.80	0.726	0.39	1.93