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### ESTIMATION OF TOMATO NUTRITIONAL STATUS BY VIS-NIR PORTABLE SPECTROPHOTOMETRIC SYSTEM

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**ABSTRACT** Determination of crop nitrogen (N) nutritional status by rapid and non-destructive methods is an essential means to optimize fertiliser-N use and minimize N losses. Spectral reflectance values of tomato leaves obtained by VIS-NIR spectrophotometry are reported to be a powerful tool for diagnosis of plant nutritional and health status. The aim of the study was to evaluate the possibility and the accuracy of the estimation of tomato leaf N content performed through a rapid, portable and non-destructive spectrophotometric VIS-NIR system, in comparison with chemical standard analyses. Nearly 2000 leaves of processing tomato grown in a field experiment with different fertiliser-N rates and forms were collected at three crop stages (from June to August 2008) and analysed for total N. The spectrophotometric acquisition was realised through an instrumental device consisting in a notebook connected to a handy punctual VIS-NIR spectrophotometer (400-1100 nm range, a bandwidth of 3 nm) and a contact probe for solids (optical diameter 3 mm, 45° diffuse illumination without specular component). Mean reflectance leaf values were compared to each reference chemical value by chemometric multivariate methods (Partial Least Squares regression analysis). The prediction ability of the model was high, being a SEP of 0.17 and RMSE of 0.14, the latter corresponding to 3.4% of the average measured values of nitrogen. The correlation between predicted and observed chemical values showed highly significant values with an  $R^2=0.97$ .

**Keywords:** Nitrogen content, Spectrophotometry, VIS-NIR, Tomato leaf, Non-destructive, SPAD, SAP test.

**INTRODUCTION** There is an increasing effort to optimize nitrogen fertilization and to improve its use efficiency of crops in order to achieve high yields with reduced N rates and limit environmental side effects related to its leaching (Neeteson and Carton, 2001; Rahn, 2002; Burns, 2006). This can be done by a fine-tuning of fertilisation rate and a dynamic N management, based on N requirements at each growth stage and adjustments

according to the actual nutritional status monitored periodically during the season (Battilani et al., 2003; Tremblay and Belec, 2005; Lemaire, 2007; Farneselli et al., 2008). This is the case of fertigated vegetables, such as processing tomato, where the fertilizer rate can be split and adjusted throughout the whole growing cycle by means of a drip irrigation system (Phene, 1999; Singandhupe et al., 2003). Therefore, the determination of the N nutritional status of a crop during the cycle is essential. The critical N concentration, which is the minimum the plant requires for its maximum growth (Greenwood et al., 1990; Lemaire and Gastal, 1997), should be the reference to evaluate if the crop is at sub-optimal, optimal or luxury consumption at any time of the cycle. Generally standard laboratory analysis of N content in the above-ground biomass are expensive and time consuming (Lemaire, 2007), especially if a rapid crop N status evaluation is required for in-season decision making procedures (Lemaire et al., 2008). For this reason, quick and practical tests have been proposed, some of which are already spread among growers. The chlorophyll meter readings (e.g. SPAD-502, Minolta) and the measurements of N-NO<sup>3</sup> concentration in petiole sap (SAP test). Both SPAD and SAP test however seem to better indicate N deficiencies than excess, therefore their use for reducing over-fertilisation could be not efficacious (Hartz, 2003). For these reasons, this study proposes a rapid, non-destructive, cost-effective technique to predict tomato leaves nitrogen nutritional status utilising a VIS-NIR (visible–near infrared) portable spectrophotometer is proposed and its results compared with results from standard chemical analyses.

## MATERIALS AND METHODS

**Data collection** The analyses were realised on a tomato crop grown in the field (Experimental Station of the Department of Agricultural and Environmental Sciences, in Papiano, Tiber Valley, Perugia province, Central Italy, 43°N, elev. 165 m) with 13 different fertilisation treatments. All treatments received the same amount of other nutrients. Nearly 2000 leaves from near 100 different plants were collected in three different periods from June to August 2008 and analysed for total N. Thirty leaves of each sample were analysed at the chemistry laboratory of the University of Perugia. The remaining 20 leaves were analyzed at the CRA-ING Laboratory using spectrophotometric techniques.

**Spectrophotometric analysis** A portable single channel spectrophotometer was used. For spectral acquisition, the ‘pen’ probe was used to measure the spectral reflectance response on each single leaf (spot area 10 mm<sup>2</sup>). The diffuse reflectance measure is referred to the light diffuse quote that is reflected by the material and acquired by an optical quartz fibre (0.7 mm in diameter) fixed at 45° inside a circular aperture of 4 mm in diameter. The material surface due to its softness was able to include all the circular aperture avoiding any external light interference. The spectral measurements were performed in laboratory considering a white calibration (small variable in function of the external light), the instrumental integration time (light acquisition time) and subtracting the background noise (variable in function of the instrument temperature). A very low Signal/Noise ratio was observed in the beginning and at the end of the spectral data, affecting the accuracy measurements, so only the spectrum in the range 400-1100 nm were taken into account for the analysis. All spectral values were expressed in terms of relative reflectance. To remove drift effect for each thesis, 12-15, the leaves were chosen randomly. After 30-35 spectral measurements a new white calibration was effectuated.

**Chemometric analysis** Mean reflectance values of all leaves for each treatment were compared to each reference chemical values by chemometric multivariate methods. The procedure includes the following steps: 1) creation of extraction of raw spectra dataset, to be used as X-block variables; 2) creation of measured values dataset to be used as reference or response variable (Y variable); 3) data fusion of the two dataset (Y and X-block) in one analysis dataset (AS); 4) random separation of the AS into two subsets, one for the model (MS=75% of AD) and one for the external validation test (TS=25% of AD); 5) application of different pre-processing algorithms to X-block and Y; 6) application of chemometric technique (Partial Least Square-PLS): modelling and testing; 7) calculation of efficiency parameter of prediction. Prediction of nitrogen leaves content was performed by Partial Least Squares (PLS) regression model, using PLS Toolbox in MATLAB V 7.0 (The Math Works, Natick, USA). Partial Least Squares is a soft-modelling method (Wold et al., 2001) for constructing predictive models when the factors are many and highly collinear. The model works through a specific algorithm (SIMPLS) on the whole array variables (input variables, X-block) and on the observed values (Y variable), after pre-processing treatments. The model determines the minimum set of the n estimation variables (LV, latent variables) by a recursive process. The model includes a calibration phase and a validation phase and for both phases it calculates the residual errors (Root mean square error in calibration RMSEC and validation RMSECV). The prediction ability of the test depends on the number of the LV used in the model and was performed by means of statistical parameters such as RMSE (Root Mean Square Error) and SEP (Standard Error of Prevision) and correlation coefficient (r) between observed and predicted values and bias. The model was chosen with a number of LV that determines the highest value of correlation between predicted and measured which presents the minimum SEP value.

**RESULTS** In Table 1 values and results of PLS model and test prediction of N content in tomato leaves from spectral reflectance analysis are reported. The best model was obtained through a Standard normal deviate (S<sub>nv</sub>) X-block pre-processing algorithm, and an autoscale Y-block pre-processing. The test resulted to be high with an r value of 0.99. Moreover the values of SEP and RMSE in the test are very low (0.14 for both) increasing the model robustness.

Table 1. Results of PLS multivariate analysis prediction of N content in tomato leaves from spectral reflectance analysis.

MODEL (75% samples randomly extracted)	
n° LV	20
Pre-processing X-block	Snv
Pre-processing Y-block	Autoscale
RMSEC	0.1696
RMSECV	0.5503
r (measured vs predicted)	0.9794
SEP	0.1706
RMSE	0.1696
TEST (25% samples randomly extracted)	
r (measured vs predicted)	0.9871
SEP	0.1447
RMSE	0.1429
Bias	0.0139

The prediction ability of the model revealed to be high, being the SEP equal to 0.17 and the RMSE to 0.14, the latter corresponding to 3.4% of the average measured values of nitrogen. The correlation between predicted values from spectral reflectance analysis and the observed chemical values showed highly significant coefficient of determination ( $R^2=0.97$ , Fig. 1).

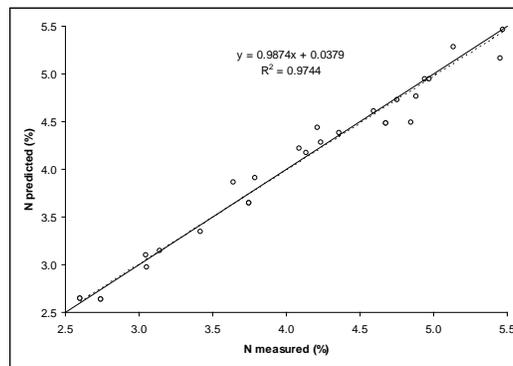


Figure 1. Correlation between measured and predicted values of N in the test (i.e. 25% of whole sample dataset randomly extracted).

**CONCLUSION** The results showed the high efficiency of estimation of leaf N content with a portable spectrophotometer by means of chemometric procedures. In this case the system allowed performing spectral measurements with an acquisition time of 2 s *per* leaf, and a power supply autonomy of 1.5 h. This time allows the sampling of 500-800 leaves that is 100-150 plants. The utilization of the proposed system, increasing efficiency, allows better knowledge of nutritional status of tomato plants, with more detailed and sharp information and on wider areas. More detailed information either in space (increase in detail) and in time (increase of the number and time of measurements)

is an essential tool to increase and stabilize crop quality levels and to optimize the nutrient use efficiency, mainly in low input production models.

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