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Innovative application of NIR-AOTF and MRI to study water behaviour in cut flowers

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Abstract: In order to study the water status of cut flowers, a comparision study was made between flowers stored in water and flowers stored «dry pack», by using a portable NIR (near infrared)-AOTF(acousto-optical tunable filter) instrument and MRI (magnetic resonance imaging). As model flower, *Zantedeschia aethiopica* (commercially known as Calla lily) was used. To predict the weight loss and water content by NIR-AOTF, cut flowers were dried in a cold room at 10°C (±1°C) and 85% (±5%) relative humidity (RH), and measured for weight loss. For MRI application, 4 and 20°C storage temperatures were used for flowers kept in water or dry; two stem sections, basal and middle, were measured. Significant correlation results for weight loss and water content (R^2 in calibration = 0.98 for estimated % of water loss and 0.96 for % of water content; R^2cv = 0.95 and 0.90) were obtained by NIR-AOTF spectra acquisitions. MRI detected vessel degradation in the stem of the water-stored flowers at 4°C but at 20°C in dry storage no vessel degradation appeared and images were correlated with dry matter values. The use of image software allowed the transformation of images in normalized population and pixel intensity, which gave hints about the potential use of these data to combine with NIR-AOTF data. NIR-AOTF, an easy-to-use and non destructive instrument, can be used to predict the vase life of cut flowers by measuring water content or weight loss. MRI is a powerful tool to identify the plugging of vessels but it is destructive; the image software used represents a useful tool to correlate MRI with NIR-AOTF to predict vessel plugging.

1. Introduction

It is well known that the vase life of cut flowers depends on water quality in terms of sanitation, nutrients and acidity regulators. Water lost during the postharvest period can normally be replaced through the vase solution. Nevertheless, desiccation is one of the most important postharvest problems for cut flowers due to the plugging of xylematic vessels (i.e. bent neck of cut roses or stem break of cut gerbera flowers), disorders caused by plugging of the cut surface of the stem due to bacteria, exudations or colloidal materials. Thus, non destructive identification of the water status in the stem of cut flowers can be useful to predict postharvest vase life. *Zantedeschia aethiopica* (commercially known as Calla lily) has long been an important cut flower, and new green-tinged and different-shaped variants are increasingly important. The flowers are normally pulled from the rhizome, and re-cut to ensure adequate water uptake (Reid, 2004). This practice is very important to guarantee a long shelf life if flowers are maintained at the correct, low temperature. The fresh weight of spathe of *Z. aethiopica* decreases with time while that of scape and spadice increases (Tjia and Funnel, 1986). A standard preservative solution used to prolong the longevity of cut flowers (8-HQC + sucrose) was deleterious to *Zantedeschia* foliage, reducing display life several fold (Skutnik *et al*., 2001). In a recent paper (Ahmad *et al*., 2013 a) it was shown that floral preservative was ineffective to prolong vase life of Calla while it was tolerant of high water pH (8.1) and vase life varied from 9.2 d for acidic solutions (pH 3.2) to 10.1 d for solutions with intermediate pH (6.3). All of these results are conditioned by the water quality and behaviour. MRI (magnetic resonance imaging) has been used to monitor the developmental change of *Zantedeschia* Spreng. tuber (Robinson *et al*., 2000) but no paper has reported, to our knowledge, its application on flower stems. NIR (near infrared) spectroscopy is an excellent technique to detect the water content inside the tissue in a non destructive way because water has a very strong signal (Cozzolino *et al*., 2006). It has been applied to measure the water potential in vine leaves (De Bei *et al*., 2011) as well as the water loss of grape berry during dehydration (Bellincontro *et al*., 2011). In the research reported here, a portable NIR-AOTF spectrometer was used to non destructively measure the water content and weight loss of Calla lily, as model cut flower, to predict its vase life;

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subsequently, MRI was applied to study the water traslocation in Calla lily stems kept at two storage temperatures (4 and 20°C). Image software was used to transform the MR images in pixel intensities for graphical representation and comparative evaluation. In subsequent investigations, the correlation between MRI results and NIR-AOTF spectra will be tested with the aim of performing a calibration model able to predict vessel plugging of flowers.

2. Materials and Methods

Material and experimental procedure

Calla lily (*Zantedeschia aethiopica*) cv. Childsiana flowers, at commercial stage of development, were picked in the morning and immediately placed in water, the stem surface was placed in sterilized water after recutting. The average weight of flowers was 80 g; color of spathe L= 75.7±2.9 a= -9.7±1.2 b= 44.5±4.5; color of stem L= 50.1±6.6 a= -8.8±0.5 b= 28.6±1.3 measured by a CM-2600d colorimeter (Konica Minolta Inc., Ramsey, NY) set at SCE (specular component excluded) measuring CIELAB coordinates L, a, and b. In order to build a calibration curve for weight loss, in the first experiment, Calla flowers (50) were kept dry, horizontally placed in cardboard boxes covered with plastic film to avoid excess air flow, in a cold room at 10°C (±1°C) and 85% (±5%) RH, with coil fans blowing during the cooling intervals. Each single flower was weighed on a scale (Cubis mod., Sartorius-Stedim Italia spa, Florence, Italy) every 8 h. Weight loss and water content were calculated and expressed as %.

NIR spectra collection

A Luminar 5030 miniature, hand-held NIR-AOTF analyzer (Brimrose Corporation, Baltimore, Maryland, USA) was used for the NIR spectra acquisitions, obtained by putting the optical sensor in contact with four sections of the flower: basal, middle, upper, and spathe. Ten spectral acquisitions were run for each section (along and all around), recorded in transmittance mode (Bellincontro *et al.*, 2011) and then averaged. A single measurement, at the speed of 16000 wavelength sec^{-1}, was conducted in the 1100-300 nm range, with 2 nm wavelength increments and 50 spectra per average, which represents a good compromise between acquisition speed and signal quality of the spectrum.

Near infrared spectroscopy analisys and chemometrics

Raw spectra were statistically pre-treated for absorbance (log 1/T) transformation using SNAP 2.03 software (Brimrose Corporation, Baltimore, Maryland, USA). The absorbance spectra, obtained as spectral average, were used as X-variables and opposite to the Y-variables (water content and water loss) in model calculation, performed by chemometric procedures of filtering and partial least square (PLS) calculation. Model validation in parameter prediction was obtained by full cross-validation (leave-one-out) method, and no outlier identification and elimination was applied. The statistical R^2 indexes (coefficient of multiple determination) in calibration (R^2_c), and in cross-validation (R^2_{cv}) were defined to establish the correlation between NIR spectra and destructive measurement. Root Mean Standard Error of Cross Validation (RMSECV) and the number of latent variables (LVs), minimizing the error in modeling, were used to determine the significance of the calculations. Finally, the RPD values, defined as the ratio between SD and SECV, were also calculated in order to define the robustness of predicting responses. This experiment was run until complete wilting of the flowers (10 days). A parallel destructive experiment was carried out: flowers (50) were kept under the same conditions and NIR acquisitions were performed in a similar way as described above; after each NIR acquisition, sections were cut and used to measure water content by drying them in an electric ventilated oven (Tecno-lab srl, Brescia, Italy) at 70°C for 72 h. Ten flowers were used for each sampling time.

MRI experiment

In the second experiment, flowers were collected and in the laboratory the stems were recut in sterilized water and then placed in the same. Flowers were divided into two lots: 60 flowers were kept in water (10 flowers per vase, 6 vases); other 60 flowers were placed horizontally, wrapped, not tightly, in plastic film as used commercially. Both lots were split and stored in two storage rooms at 4 or 20°C (±1°C) at 70% (±5%) RH. The experiment lasted 12 days. Dry matter was measured by using a ventilated oven as described above, at each sampling time. MRI measurements were performed on flower sections at time 0, after 3 and 12 days. At each sampling time, MRI analyses were performed on two sections (15 cm long) of the stem, cut from 5 cm above the stem cut surface and from 15 cm below the spathe, named basal and middle, respectively. A Bruker AVANCE 300 MHz spectrometer (Bruker Biospin Corp, Bilerica, MA, USA) equipped with cylindrical birdcage single-tuned nucleus (^{1}H) coil probehead with an inner diameter of 20.0 mm was used (Taglienti *et al.*, 2009). The water signal was monitored and used for the image reconstruction. Gradient-Echo (GEFI) and Multi-Slice-Multi-Echo (MSME) experiments, m_gefi_ortho and m_msme_ortho, respectively (Bruker library), were performed according to standard procedures. In GEFI measurements, which generate echoes by applying gradient pulses, the field of view was 20.0 mm x 20.0 mm, the matrix size 128 x 128 pixels and spectral width 100.0 kHz. The echo and repetition times were set equal to 2.445 ms and 60.0 ms, respectively. The number of scans was 1; slice thickness was 1.01 mm; and excitation pulse was a sinc3. The data were processed to obtain images 128 x 128 in size and a field of view of 20.0 mm x 20.0 mm. The processing mode was FT_MODE, the latter was complex_FFT and spikes elimination was allowed. In MSME experiments, which produce echoes via a spin-echo-based sequence, the field of view was 20.0 mm x 20.0 mm; the

matrix size 128 x 128; spectral width 100.0 kHz; the echo and repetition times were set equal to 17.5 ms and 6000.0 ms, respectively; the number of echoes and images were 196; number of scans and dummy scans was 1; slice thickness was 1.0 mm; and the excitation pulse was a sinc3. The data were processed to obtain images 128 x 128 in size, a field view of 20.0 mm x 20.0 mm, the processing mode FT_MODE, the latter complex_ FFT and with spikes elimination allowed. The intensity decay of the NMR signal *vs*. the echo time was calculated by the Bruker software ParaVision 3.0.2 in order to calculate the local transverse relaxation time, i.e. T_2. GEFI and MSME images were also analysed by ImageJ 1.41 (Rasband, 2007) which allows for greyscale analysis. For GEFI experiments, the axial image was analysed by ImageJ software, while for MSME the intensities of the 24th acquired image were reported to a scale from 0 to 255, the first value corresponding to full black colour and the second to complete white. The whole sample was selected, and parameterised images were used to create curves according to the pixel intensities.

3. Results and Discussion

The absorbance mean spectra of the acquisitions in the floral sections showed characteristic molecular references of the absorbance peaks (data not shown). Spectra were characterized by two principal water absorption bands around 1450 nm and 1920-1950 nm (Nicolai *et al*., 2007). These bands are assigned to the first overtone of the symmetric and asymmetric OH stretching and/or combination bands (1450 nm), and to the combination of the OH stretching band and to the OH bending band (1920-1950 nm), respectively (Shenk and Westerhaus, 1996). This spectral response is strictly correlated with the floral water content; thus the weight loss estimation, mainly due to water loss, is predictable. In Table 1 analytical measurements of weight loss (%) and water content (%) are statistically defined by descriptive indexes. Data range (as min and max values), mean, and SD (standard deviation) are reported to describe the variability of the data sets destined to the multivariate calibration models. For regression models, different pretreatments were tested on the spectra sets (data not shown) previously transformed in absorbance (log 1/T). First derivation by Savitzky-Golay filter (11 points of smoothing, 2nd order) proved to be the best performing and were used in subsequent chemometric applications and regressive PLS1 calculation. Calibration and cross-validation results for the models obtained from the two tested parameters, are reported, as characteristic scatter plots of multivariate regressions, in Figure 1. Significant correlation results were achieved for both models: the determination coefficients (R^2) in calibration were 0.98 and 0.96 for the estimated percentage of the weight loss and the estimated percentage of the water content, respectively; the coefficients of determination in cross-validation (R^2cv) were, respectively, 0.95 and 0.90. For the estimation of the predictive accuracy of the models, an R^2cv greater than 0.9 represents a valid quantitative information (Maeda *et al*., 1995). Cross validation is a practical method to demonstrate how NIRS can predict a qualitative attribute, even if it would be better to estimate the accuracy of the application by using an appropriate, preferably external, test or validation set (Dardenne, 2010). In leave-one-out cross validation, one sample is removed from the dataset and a calibration model is built on the basis of the remaining subset, using that samples to calculate the residual prediction (Cozzolino *et al*., 2011). The significant results obtained in terms of correlation on the predictive models can also be attributed to the high degree of accuracy and precision of the reference data (Bellincontro *et al*., 2012).

Table 1 - Statistical analyses of sample sets relative to the two analyzed parameters (weight loss and water content) during the first experiment

Parameter	Samples	Mean	SD	Min	Max
Weight loss (%)	95	7.95	4.45	1.51	19.60
Water content (%)	75	93.11	3.31	93.11	97.11

Mean, standard deviation (SD), range (min. and max.) are reported expressed as %. Number of sample s (i.e. the number of destructive measurements carried out) is reported

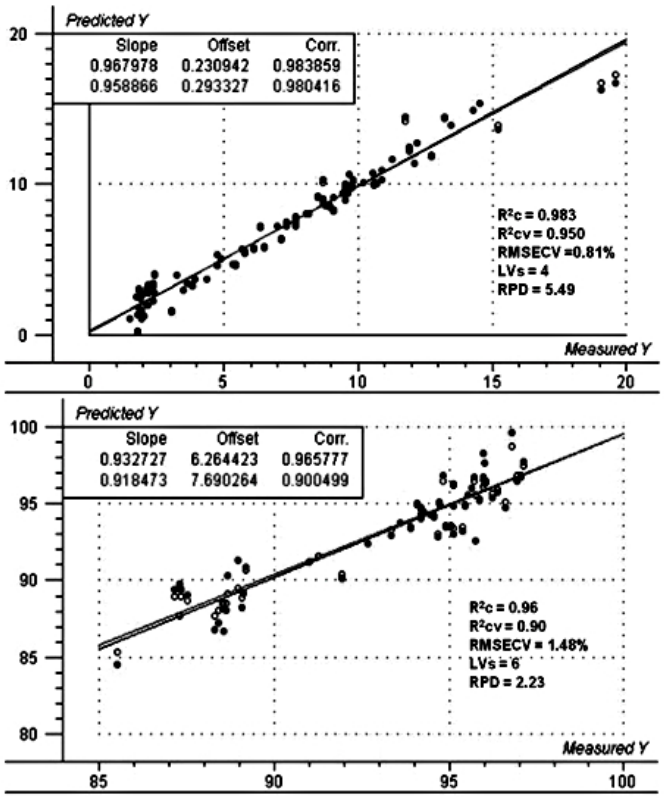

Fig. 1 - Scatter plots relative to the prediction models for the percentage of weight loss of flowers (top). Scatter plots relative to the prediction models for the percentage of water content of flowers (bottom). Measured values are plotted versus predicted values and significant indexes of calibration and validation are reported.

However, the real and applicative performance of the predictive models is better defined if combined with the estimation indexes referring to potential errors in calibration and prediction or cross-validation (RMSEC, RMSEP and RMSECV). Here we report RMSECV (root mean standard error in cross-validation) which was 0.81% and 1.48% in predicting the flowers' weight loss and water content, respectively. Quite low LVs values were obtained for both PLS models (Fig. 1), and it is possible to observe that a small number of latent variables could reduce the possible errors in predictive responses of the models. RPD (Ratio of Performance to Deviation) indexes are also reported in figure 1. The RPD ratio is another statistical index useful for evaluating the predictive ability of the NIRS and is calculated as the ratio between SD of destructive measurements and the standard errors of prediction. A RPD below 2.5-3 means that the model has low ability of discrimination from high values of the response variable; values above 5 indicate good discrimination, especially if destined for quality control (Williams and Sobering, 1996).

As regards the second experiment on the use of MRI, the dry matter in the basal (cut surface) and middle sections were similar at the start of the experiment (Table 2). After 3 days, at 4°C, no differences were observed among samples, whatever the location of the section or the method of storage (dry or water). At 20°C, after 3 days, the dry matter was similar (4.7 and 4.9%) in the middle sections regardless of the storage method and similar to the one at 4°C while, the basal sections had 3.4 and 4.1% of dry matter in flowers kept in water or dry, respectively; after 12 days the values significantly decreased, 3.7 and 2.8%, in the middle and basal sections, respectively, of flowers kept in water. The results for dry matter indicate that storage temperature plays an important role in preserving the integrity of the stem as has been reported recently for *Lilium* (Prisa *et al*., 2013) and also to maintain fresh and dry weight (Ahmad *et al*., 2013 b). Dry matter was also affected by storage method: storage in water preserved the integrity of the internal structure of the Calla stem. Dry matter concentration is mainly due to sugars concentration which are known for their importance in vase life of cut flowers. The significant reduction of dry matter in middle and basal sections of the flowers kept in water at 20°C might be due to the presence of bacteria. Bacteria can enter maintenance solutions from the external surface of the flowers (Teixeira da Silva, 2003). In tap water, *Acinetobacter* sp., *Bacillus pumilus* and *Pantoea agglomerans* cells moved from the outside to inside the flower parts, becoming endophytic bacteria with a role in stem break of gerbera flowers (Balestra *et al*., 2005). Xylematic vessel blockage could be due to some amorphous or physiological deposition and rod-shaped bacteria located within the 5 cm stem end of the cut flower as shown by scanning electron microscope (Wang *et al*., 2014). A partial relationship between the lower content of dry matter and the tissue degradation seems to be confirmed by MRI. In figure 2, degradation of the cut stem surface (white area indicates that water moves freely) is clear, while the middle section still maintains intact tissue and its vessels are

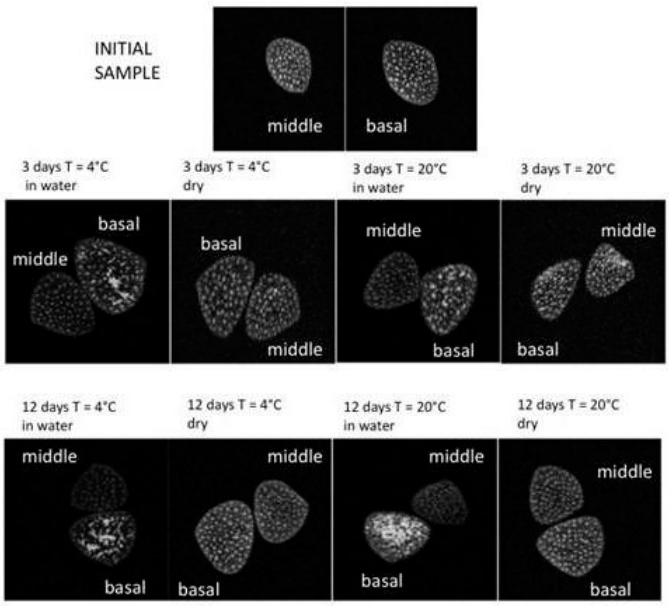

Fig. 2 - MSME MRI images of flowers, middle and basal sections. On top, initial samples. Ten images were taken for each sample at each sampling time. The reported images are representative of the pool of 10 for each sample, which showed similarity of behaviour.

Table 2 - Dry matter content (%) of sections (basal and middle) of Calla lily flowers kept at 4 or 20°C, dry or in water

	Water M.S.	Dry M.S.	Water B.S.	Dry B.S.	Middle section	Basal section
Initial time					4.8±0.3 bcd	4.4±0.4 cd
4°C						
After 3 days	5.2±0.3 ab	5.2±0.2 ab	4.7±0.4 bcd	4.7±0.4 bcd		
After 12 days	4.9±0.4 abc	4.9±0.5 abc	4.9±0.4 abc	4.9±0.5 abc		
20°C						
After 3 days	4.7±0.4 bcd	4.9±0.4 abc	3.4±0.4 ef	4.1±0.2 de		
After 12 days	3.7±0.2 e	4.5±0.4 cd	2.8±0.2 f	4.3±0.2 cd		

Data are the mean of 10 flowers at each sampling time ± SD. Values with different letters are significantly different ($p<0.05$) by LSD. (M.S.= medium section; B.S.= basal section).

visible (white dot); this image is very clear in the initial samples. At 4°C in water, the degradation is less diffuse than at 20°C, both in the basal and middle sections. These images do not reflect the data of dry matter probably because the lower temperature reduces the consumption of sugars by respiration. In contrast, similarity of the images between the initial samples and the samples kept dry at 4 or 20°C is evident, in agreement with dry matter data. In water at 20°C, the images show a great degradation which is in line with the data of dry matter loss.

Elaboration of the images in terms of normalized population *vs* pixel intensity, in order to have numbers to combine with the NIR data, made it possible to emphasize that the basal sections of the flowers kept in water, not only at 20°C but also at 4°C, are contaminated already in the first 3 days of vase life. The GEFI image (MR signal prosectional to water content) of the basal section of flowers kept at 20°C, at a pixel intensity of 100, shows how great the difference is (0.2 vs 0.05-0.1 normalized population) between the initial sample (black line) and the lines (red and bleu) of the flowers kept in water, at 3 and 12 days (Fig. 3 a). This result pinpoints a greater water content in the basal tissue than in the middle section where the difference is not evident (data not shown). The same images elaborated as MSME signal (MR signal prosectional to water mobility) (Fig. 3 b) show a shift of the red and blue lines (flowers in water at 3 and 12 days, respectively) toward the left (Y axis), compared to the other samples, and a particular peak at 200 pixels (which suggests a change in water mobility) similar to the one observed in the GEFI image (water content) but at 100 pixels (Fig. 3a). Both these results point out that a change in water content and mobility occurs; this event could be attributed to the presence of bacteria. The curve pattern of the middle section is similar but with a greater distance between the two pairs of lines (red and bluu vs green and light blue) and no peak is observed at 200 pixel (Fig. 3 c). The peak of the normalized population for red and blue lines is at 25 pixels while for green and light blue lines it is between 50 and 75 pixels, meaning a clearer image, as can be observed in Figure 2. The patterns of curves of basal and middle sections are very different, especially for red and blue lines: at 50 pixels the value of

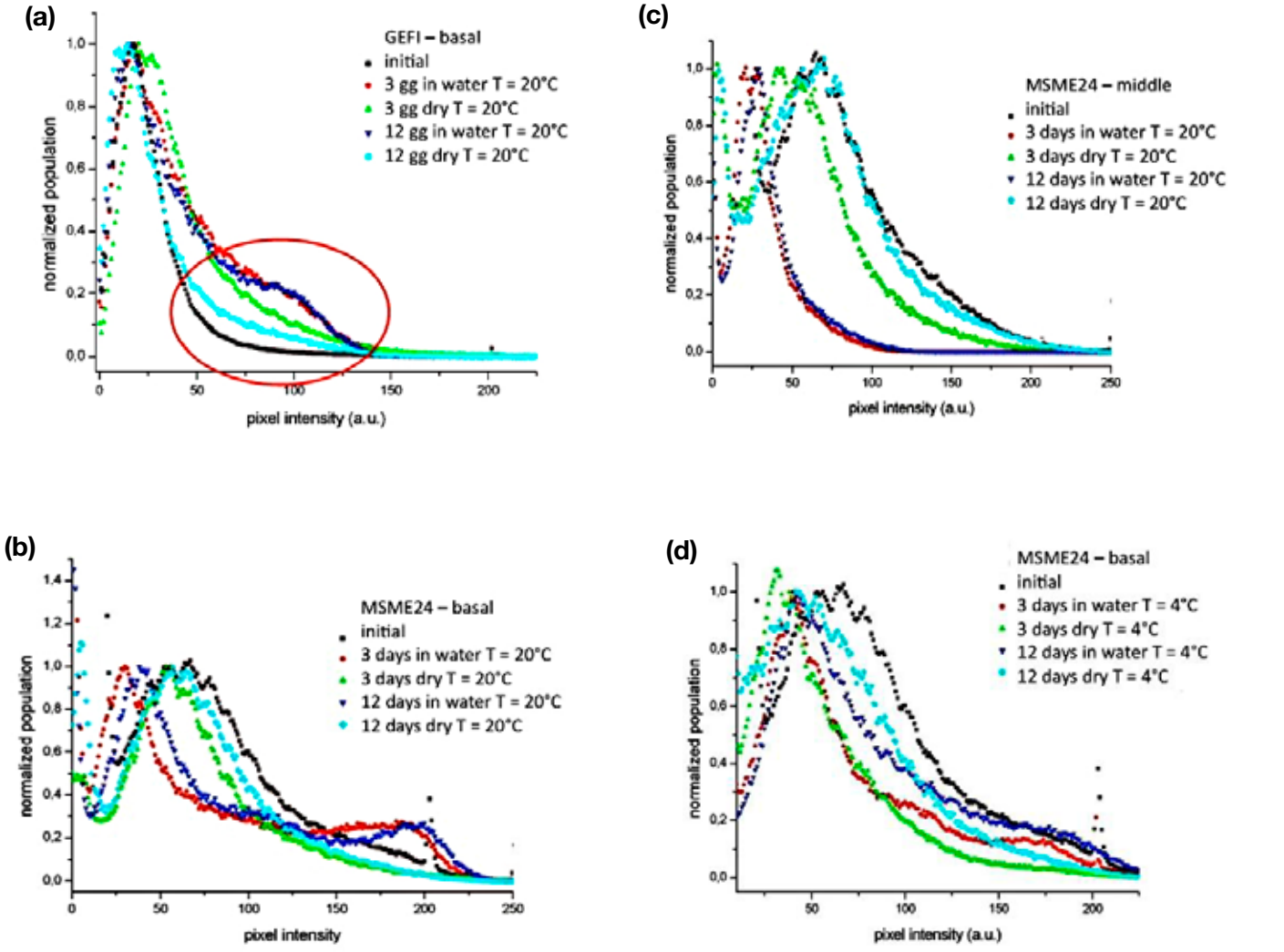

Fig. 3 - Normalized population vs pixels (a.u. = auxiliary units) elaboration of GEFI (3a) and MSME (3b,c,d) images.

the basal section is 0.4 normalized population while the one of the middle section is 0.2; for the green and light blue lines (dry flowers at 3 and 12 days) the 100-pixel values are similar for the basal and middle sections. Thus, for flowers that were kept dry, clearer images and less difference in the images of the sections can be noted.

At 4°C, the basal section showed coupled lines on the basis of sampling times, with a shift towards the Y axis, compared to the black line (initial sample): the peak of the normalized population of the initial sample is around 75 pixels, while for the rest of the lines it is between 25 and a little bit more than 50 pixels (Fig. 3 d). In the range 50 and 100 pixels, the green and red lines, which refer, respectively, to dry and water samples at 3 days, had lower values than the bleu and the light bleu lines, referring to water and dry samples but at 12 days. Even in this case, at 150 pixels an increase of the values of normalized population of the red and blue lines was observed as was shown at 20°C, in line with the presence of degraded tissue. In the middle section, the cited increase at 150 pixels is not visible and the pattern of lines is more confused (data not shown).

4. Conclusions

Application of the portable NIR-AOTF instrument to the stem of cut flowers (Calla lily in the present study) to non-destructively measure weight loss and water content, especially for dry shipped flowers, can be very useful to predict vase life. MRI is a very powerful tool to study the water status in the xylematic vessels and the use of of ImageJ software permits transformation of the image into numbers (pixel intensity or normalized population). Unfortunately the cost of the instrument, which depends on magnet size, makes commercial use of the instrument impossible. The use of software will permit correlation of the pixel intensity or normalized population data to spectra values of other non destructive instruments, such as the NIR-AOTF as will be examined in our future research.

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