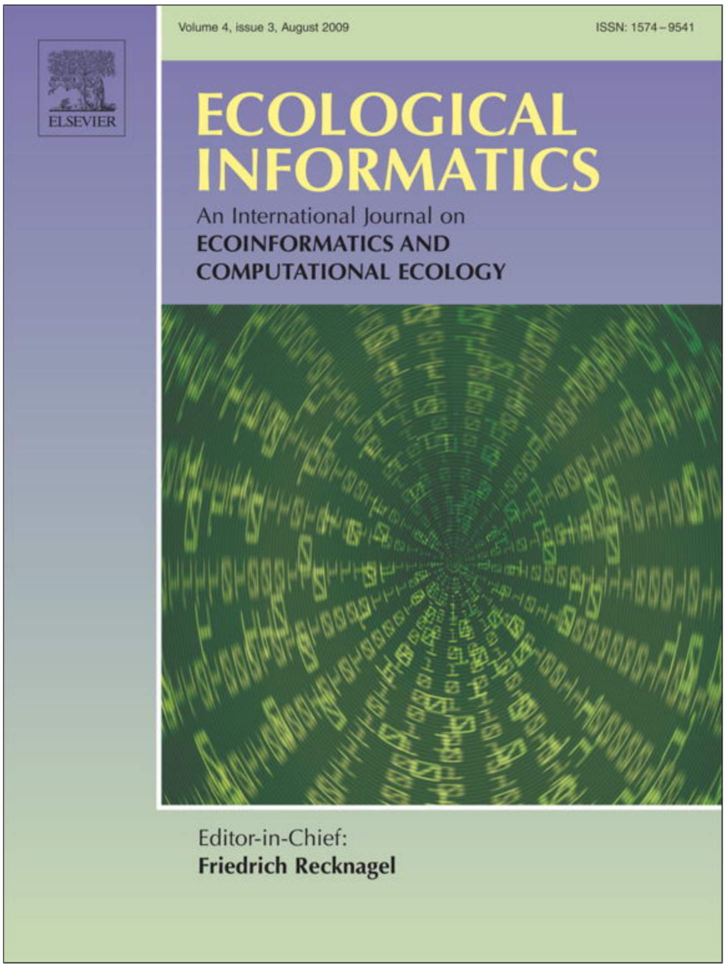
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Classification of leaf epidermis microphotographs using texture features

Elio Ramos [a,*], Denny S. Fernández [b]

[a] Department of Mathematics, University of Puerto Rico at Humacao, CUH Station, Humacao, Puerto Rico, 00791-4300
[b] Department of Biology, University of Puerto Rico at Humacao, CUH Station, Humacao, Puerto Rico, 00791-4300

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ABSTRACT

We present the results of a Gray Level Co-occurrence Matrix (GLCM) analysis for two sets of leaf epidermis images for the adaxial (20×_H) and abaxial sides (20×_E). The leaves were collected from a dry forest in Mona Island which is located between the Dominican Republic and Puerto Rico. For each set of images (GLCM) texture features were calculated namely the energy, correlation, contrast, absolute value, inverse difference, homogeneity, and entropy. From the calculated statistics a features matrix was obtained for each image and randomly divided into training set and test set using the hold-out method. In this method 70% of the images were considered as a training set and 30% as the test set. For each training and test set a linear discrimination analysis (LDA) was performed resulting in a average correct classification percent of 90% for the abaxial side in comparison with 80% for the adaxial side.

1. Introduction

Classification is one of the main tasks in basic science. In biological sciences it is the basis for the categorization of groups and the discrimination between species. Within the broad area of artificial intelligence and machine learning the field of pattern recognition aims to the automatic (unsupervised) or semiautomatic (supervised) classification of objects based on the statistical patterns observed (Tuceryan and Jain, 1998). These methodologies play an important role in such applications like biomedical image analysis, automated visual inspection, automated image retrieval, and remote sensing. Texture is one of the most useful and important characteristics for the recognition of images. The distinction between textures can be associated to differences in the intensity of the pixels and the spatial relations between them. These textures are strongly dependent on the spatial scale as is the common observation that a smooth texture at a very large scale becomes a rough texture at a small scale.

A particular texture in an image is characterized by the invariance of certain local attributes that are periodically (or quasi-periodically) distributed over a region. There are many approaches for the quantitative characterization of textures in images. A set of statistical features (contrast, entropy, homogeneity, etc.) can be calculated from a gray level co-occurrence matrix (GLCM) (e.g. Haralick et al., 1973). Other statistical approaches involves Markov Random Field models to characterize textures (Kashyap et al., 1982), measurements in a Fourier domain (Unser, 1986), and wavelets methods (Laine and Fan, 1993).

In this paper, we present the results of a texture analysis for two sets of images of microphotograph of replicas of leaf epidermis using the GLCM approach. The images present a wide variety of textures characterized by different cell structures, spatial patterns, and stomata configurations. The main objective of this paper is the development of a methodology for the supervised classification of leaf epidermis images based on the GLCM Haralick features. This investigation is a contribution to develop an automatic procedure for preliminary classification of leaf epidermis present in the rumen and/or fecal material of large herbivores, to study their diet and the possible effects on the plant communities. In the next section we describe the data sets utilized in the analysis, followed by the methodology for the statistical characterization of textures. Then, the results of the data analysis are presented.

2. Data sets and filtering

The data set consisted of two sets of images (microphotographs) of leaf epidermis of 1600 × 1200 pixels at 200× magnification each group corresponding to the abaxial (lower) and adaxial (upper) side of the leaf. The adaxial set (20×_H) consisted of 39 images and the abaxial set (20×_E) consisted of 69 images. The original 32-bit RGB images were converted to 8-bit gray scale images and enhanced using a histogram stretching method. Each image was identified visually and an acronym was assigned based on the abbreviated scientific name of the identified species. Furthermore, the amount of images was reduced by considering only species with images available for both sides (adaxial and abaxial). This resulted in two smaller samples containing 35 images. Given the large size of the original images and the relatively small quantity in each group (for classification purposes) each images was cropped into 16 equal size regions (prototypes) of 400 × 300 pixels. In both cases this resulted in 560 images for the 20×_E and the 20×_H set. Examples of image

* Corresponding author.
E-mail address: eramos@mate.uprh.edu (E. Ramos).

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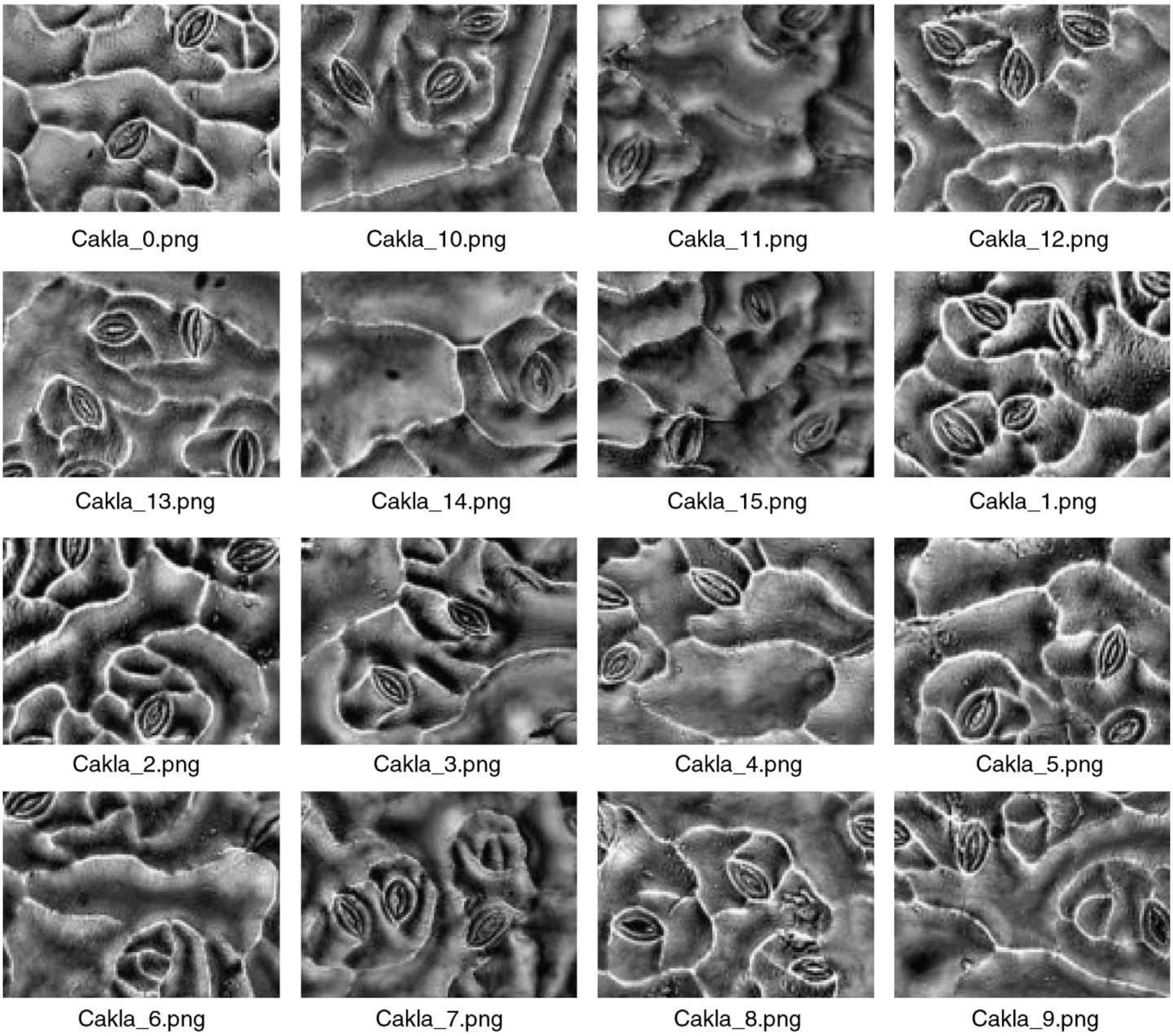

Fig. 1. Abaxial (20×_E) prototypes for the species Cakile lanceolata (Caklan).

prototypes for the species *Cakile lanceolata* are shown in Fig. 1 (abaxial) and Fig. 2 (adaxial).

Visual examination of the regions indicated the presence of noisy images with a high level of distortion and visual artifacts. For this purpose an automatic method was developed to select the best prototypes by computing the standard deviation of the average gray level value of each prototype image. With this approach the prototypes with the lowest or highest standard deviations corresponded to images of low or high dispersion among the prototypes and consequently the best or worst quality for classification purposes. The images were sorted from the lowest to the highest dispersion and the top 15 prototypes were selected for the analysis. This resulted in two samples of 240 images for the 20×_E and 20×_H data sets.

3. Texture features characterization

The classification of the images was based on the texture features observed in the images. Although there is no formal definition of texture it is recognized as one of the most important sources of information in the visual perception of humans. In general terms, texture is related with the statistical distribution of gray tones. The corresponding distribution may result in the perception of textures as being fine, coarse, or smooth and more complex visual perceptions like irregularity, complexity, and rippled patterns.

The Gray Scale Co-occurrence Matrix (GLCM) is a tabulation of how often different combinations of gray levels occurs in an image (e.g. Haralick et al., 1973) and is commonly referred as a two-dimensional histogram where unlike the one-dimensional version it counts the pixels intensities by pairs. Formally, for an image g of G gray levels we can construct a $N \times N$ gray level co-occurrence matrix $M_{d,\theta}$. The elements of $M_{d,\theta}$ represent the probability of the co-occurrence of gray values i, j at points p_1, p_2 separated by distance d and angle θ:

$$M_{d,\theta} = \mathrm{Prob}\{g(p_1) = i, g(p_2) = j\} \quad (1)$$

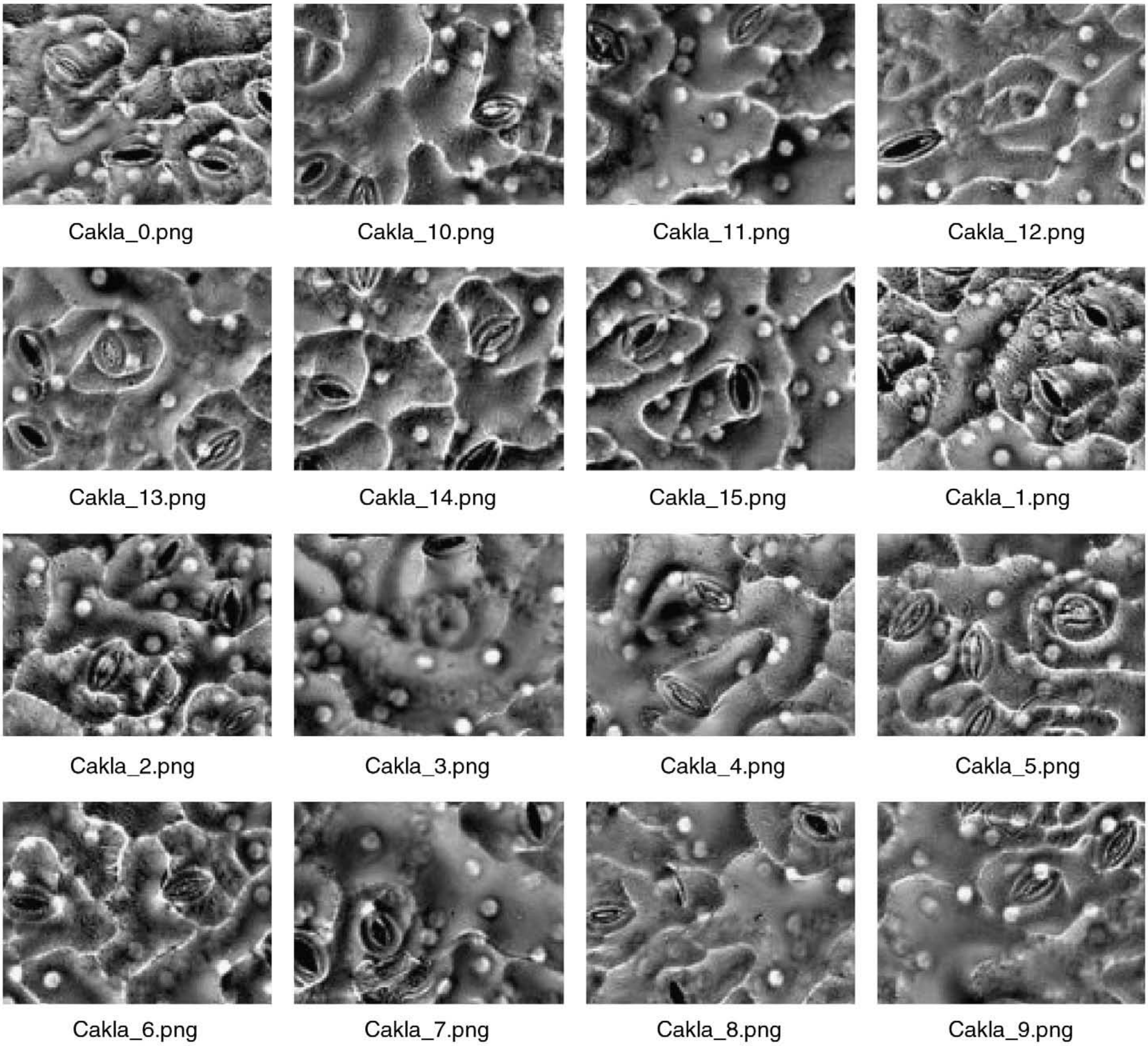

Fig. 2. Abaxial (20×_H) prototypes for the species Cakile lanceolata (Caklan).

given that $d(p_{1,} p_2)=d$ and $\angle(p_{1,} p_2)=\theta$, where $p_i=(x_i, y_i)$, and $i=1, 2$ and

$$d(p_1,p_2) = \sqrt{(x_2-x_1)^2 + (y_2-y_1)^2} \quad (2)$$

$$\angle(p_1,p_2) = \arctan((y_2-y_1)/(x_2-x_1)) \quad (3)$$

In general the $M_{d,\theta}$ matrix is symmetric and the elements of the diagonal quantify the amount of pixel pairs with the same gray value. Consequently, higher values, near the diagonal of the matrix, correspond to images with low contrast. On the other hand, elements far away from the diagonal represent pixels pairs with different gray values and consequently correspond to images with a higher contrast. Once the $M_{d,\theta}$ matrix is calculated for an image a wide variety of texture features can be calculated (detailed formulas are presented in the Appendix A).

As is shown in Eqs. (1)–(3) the calculation of the GLCM (and the texture features) requires the specification of a distance (d) and an angle (θ) between the pixels (Fig. 3). The optimum distance is dependent on the concrete set of images and texture patterns under consideration. Nevertheless, the distances commonly considered in the literature (Conners et al., 1984) are $d=1, 2, 3, 4$ and the angles $\theta=0°, 45°, 90°, 135°$. For a given image that may imply a large amount of features corresponding to all the distance and angle combinations and in practice the number of features can be reduced by the calculation of an isotropic GLCM given by:

$$M_{d,\mathrm{isotropic}} = \frac{1}{N_\theta}\sum_\theta M_{d,\theta} \quad (4)$$

where N_θ is the number of possible angles (e.g. 4). Similarly we can define a range GLCM by taking the difference between the maximum and minimum element of the matrix:

$$M_{d,\mathrm{range}} = \max(M_{d,\theta}) - \min(M_{d,\theta}) \quad (5)$$

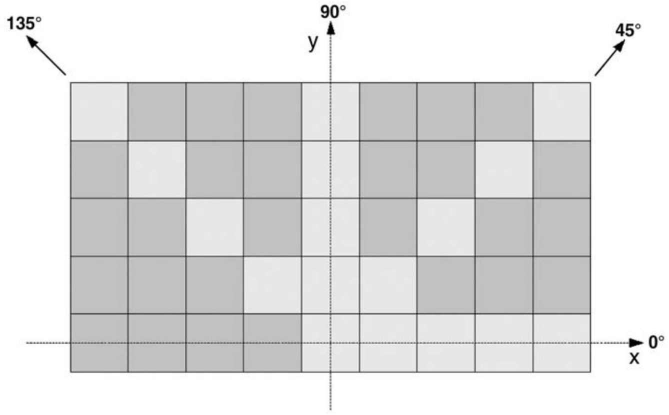

Fig. 3. Geometry for the Calculation of $M_{d,\theta}$ at four angles and four distances.

Using these two matrices we can compute isotropic texture features and the range texture features by the specification of a distance.

4. Implementation

The GLCM method was implemented as a plugin with the ImageJ public domain Java image processing software. The plugin, `GLCM.java` was developed in Java and based on a texture analysis plugin developed in NIH (Cabrera, 2005). The original texture analysis plugin source was extended by adding some additional features and modified to handle a large amount of images. Additional modules for post-processing (filtering and classification) were developed using the R programming language (Venables and Ripley, 2003).

5. Results and discussion

For each data set of 240 images (20×_E and 20×_H) 7 GLCM texture features were calculated corresponding to distances from 1 to 4. This resulted, in a matrix of 240 rows (objects) and 7 columns (features). Due to the differences in scale for each texture feature, each column was normalized to zero mean and unit variance using the transformation $f_{\mathrm{norm}} = (f - \bar{f}) / \sigma$. The normalized matrix was randomly divided into a training set and a test set using the hold-out validation approach (Theodoridis and Koutroumbas, 2006). Using this approach 70% of the rows were randomly selected as the training set and 30% of the rows were selected as the test set. Then, a multi-class linear discriminant analysis (LDA) (Fisher, 1936) was applied to each test-training pair and a correct classification percent was calculated. The process was repeated N times (e.g. $N = 100$) and a mean classification percent was obtained. The results of this analysis, for each of the data sets, are summarized in Table 1. This results show the poor performance of the classification method for a single inter-pixel distance. For the 20×_E set the highest correct classification percent (76%) is obtained from features calculated from the range GLCM at a distance of 1 pixel. Meanwhile, in the 20×_H set the highest percent (67%) is obtained from features calculated from isotropic GLCM at either 1 or 4 pixels of separation. These results are consistent with authors that suggest (e.g. Haralick et al., 1973) that the best discrimination capabilities of texture features, derived from the GLCM, are obtained by combining features from two (or more) inter-pixel distances. The results of these calculations are presented in Table 2 where the correct classification percent was obtained for several combinations of inter-pixel distances. This results show a dramatic improvement in the performance of the classification. In the case of the 20×_E set the highest percent (90%) was obtained from the range GLCM features from almost all the inter-pixel distance combinations. Meanwhile, in the case of the 20×_H set the highest percent (80%) was obtained from isotropic GLCM features from 1,3 inter-pixel distances combinations. Furthermore, the performance of the classification, by using textures features at the four distances (1,2,3,4) show a 2% improvement in the correct classification percent. However, we did not find a significant improvement of correct classification percent for distances larger than 4 pixels.

Table 1
Results of LDA classification for a sample of 240 images.

Distance	Percentage of correct classifications for leaf epidermis images			
	20×_E		20×_H	
	Isotropic GLCM	Range GLCM	Isotropic GLCM	Range GLCM
1	67	76	63	67
2	38	73	62	64
3	39	75	63	63
4	37	74	67	66

For each image a set of 8 texture features were considered at specific inter-pixel distance.

Table 2
Results of LDA classification for a sample of 240 images.

Distances	Percentage of correct classifications for leaf epidermis images			
	20×_E		20×_H	
	Isotropic GLCM	Range GLCM	Isotropic GLCM	Range GLCM
1,2	75	90	79	73
1,3	75	90	78	75
1,4	75	90	80	73
2,3	46	89	77	74
2,4	47	90	77	74

For each image a set of 16 texture features were considered for two inter-pixel distance combinations.

6. Summary and conclusions

A methodology is presented for the filtering, processing, analysis, and classification of images based on texture features derived from a Gray Scale Co-ocurrence Matrix (GLCM) Specifically, a texture analysis was carried out on two set of images of microphotographs of leaf epidermis corresponding to the abaxial (20×_E) and adaxial (20×_H) sides. Seven texture features were calculated from isotropic GLCM and a range GLCM at four inter-pixel distances. Our results indicate that the highest percent of correct classifications (90%) is obtained from the abaxial set where the features are derived from a range GLCM. On the other hand, the adaxial set results in a relatively smaller correct classification percentage (80%) from texture features derived from the isotropic GLCM. Our results indicate that the combination of texture features from separations of more than 2 pixels improves dramatically the discrimination capabilities of the method.

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Appendix A. Haralick texture features

For an image g of G gray levels Haralick (e.g. Haralick et al., 1973) proposed several texture features that can be calculated from weighting the $M_{d,\theta}$ matrix. But first, several descriptive statistics need to be calculated:

$$\mu_x = \sum_{i=0}^{G-1} i M^x_{d,\theta}(i)$$

$$\mu_y = \sum_{j=0}^{G-1} j M^y_{d,\theta}(j)$$

$$\sigma_x^2 = \sum_{i=0}^{G-1} (i-\mu_x)^2 M^x_{d,\theta}(i)$$

$$\sigma_y^2 = \sum_{j=0}^{G-1} (j-\mu_y)^2 M^y_{d,\theta}(j)$$

where

$$M^x_{d,\theta}(i) = \sum_{j=0}^{G-1} M_{d,\theta}(i,j)$$

$$M^y_{d,\theta}(j) = \sum_{i=0}^{G-1} M_{d,\theta}(i,j)$$

From the previous parameters seven features can be defined to measure different aspects of the texture information in the images:

- Energy

$$f_1 = \sum_{i,j=0}^{G-1} (M_{d,\theta}(i,j))^2$$

- Correlation

$$f_2 = (\sum_{i,j=0}^{G-1} ijM_{d,\theta}(i,j) - \mu_x\mu_y) / \sigma_x\sigma_y$$

- Contrast

$$f_3 = \sum_{i,j=0}^{G-1} (i-j)^2 M_{d,\theta}(i,j)$$

- Absolute value

$$f_4 = \sum_{i,j=0}^{G-1} |i-j| M_{d,\theta}(i,j)$$

- Inverse difference

$$f_5 = \sum_{i,j=0}^{G-1} M_{d,\theta}(i,j) / (1 + (i-j)^2)$$

- Homogeneity

$$f_6 = \sum_{i,j=0}^{G-1} M_{d,\theta}(i,j) / (1 + |i-j|^2)$$

- Entropy

$$f_7 = \sum_{i,j=0}^{G-1} M_{d,\theta}(i,j) \log_2 (M_{d,\theta}(i,j))$$

This features can be equally calculated from the isotropic (Eq. (4)) and range (Eq. (5)) matrices defined in the main text.

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