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Towards Discrimination of Plant Species by Machine Vision: Advanced Statistical Analysis of Chlorophyll Fluorescence Transients

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Abstract Automatic discrimination of plant species is required for precision farming and for advanced environmental protection. For this task, reflected sunlight has already been tested whereas fluorescence emission has been only scarcely considered. Here, we investigated the discriminative potential of chlorophyll fluorescence imaging in a case study using three closely related plant species of the family *Lamiaceae*. We compared discriminative potential of eight classifiers and four feature selection methods to identify the fluorescence parameters that can yield the highest contrast between the species. Three plant species: *Ocimum basilicum, Origanum majorana* and *Origanum vulgare* were grown separately as well as in pots where all three species were mixed. First, eight statistical classifiers were applied and tested in simulated species discrimination. The performance of the Quadratic Discriminant Classifier was found to be the most efficient. This classifier was further applied in combination with four different methods of feature selection. The Sequential Forward Floating Selection was found as the most efficient method for selecting the best performing subset of fluorescence images. The ability of the combinatorial statistical techniques for discriminating the species was also compared to the resolving power of conventional fluorescence parameters and found to be more efficient.

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Acronyms and symbols

CCD	Charge Coupled Device
ChlF	Chlorophyll Fluorescence
F_0	minimal fluorescence level of dark adapted plants when primary quinone accepter (Q_A) of Photosystem II is oxidized
F_M	maximal fluorescence level of dark adapted leaves measured when Q_A and the plastoquinone pool are reduced
$F_V=F_M-F_0$	variable fluorescence
FLDC	Fisher's Linear Discriminant Classifier
IFS	Individual Feature Selection
k-NN	*k*-Nearest Neighbor Classifier
LDC	Linear Discriminant Classifier
NEURC	Automatic neural networks Classifier
NMC	Nearest Mean Classifier
NN	Nearest Neighbor Classifier
NPQ	Non-Photochemical Quenching
QDC	Quadratic Discriminant Classifier
qP	Photochemical quenching
Rfd	Fluorescence decrease ratio
SBS	Sequential Backward Selection
SFFS	Sequential Forward Floating Selection
SFS	Sequential Forward Selection
SVC	Support Vector Classifier

Introduction

Species discrimination is applied in precision farming for machine weeding or to reduce excess use of agrochemicals

by a targeted application [1, 2]. Weed detection in crop fields and a targeted application of agrochemicals can save 50–90% of herbicides. Such a reduction would lead to significant economic and ecologic benefits [1, 3, 4]. Several methods based on machine vision of reflected natural light are available for the discrimination of plant species in field [5–7]. One class of techniques discriminates the species using their shape, size, and image texture [8, 9]. These techniques have several limitations, e.g., due to shapes that vary greatly with viewing angle, overlapping leaves that are hard to resolve, and segmentation that can be difficult due to non-uniform illumination [10]. The main disadvantage of shape-based techniques is low performance in real time [11].

Other methods utilize differences in the spectrum of the light that is reflected from plants. Earlier, the spectral resolution was limited by use of a multispectral reflectance sensor that measured the reflectance images only within several wide spectral bands [12]. With such a limited spectral resolution, non-uniform light illumination that often occurs in field conditions can greatly complicate species discrimination [13, 14]. Later, hyperspectral reflectance sensors were developed that measure hundreds of images, each in a very narrow band of the reflected light. However, the classification accuracy remained frequently inadequate even with the hyperspectral resolution (see [15], reviewed in [16]).

Rather than using reflected light, we focus here on actively excited chlorophyll fluorescence (ChlF) emission, the reporter signal that has already proven its potential both in research and in numerous applications [17, 18]. The imaging variant of the technique measures hundreds of ChlF images capturing fluorescence transient that occurs in response to actinic light exposure [18]. Many conventional ChlF parameters have been identified that have physiological interpretation and are useful for, e.g., assessment of plant health status and early detection of biotic and abiotic stresses [19–21]. We suggest that the information in the ChlF transients can be also used for plant species identification [22, 23].

Practical application of the species discrimination based on hyperspectral reflectance or on fluorescence emission imaging is limited by potentially long time intervals that may be required to collect and analyze data in real time [22, 24, 25]. The long acquisition time required to capture some of the conventional fluorescence parameters (e.g., NPQ or Rfd), [26] must be solved by development of alternative experimental protocols and/or by use of ChlF parameters that can be acquired in real time and fast enough for the particular application.

In the present work, we focus at the computation time that can be reduced by eliminating redundant or low-contrast information and by selective use of information-rich images as identified by feature selection methods [27, 28] or by genetic algorithms [23, 29, 30]. Recently, we applied a combination of the *k*-Nearest-Neighbor (*k*-NN) classifier and the Sequential Forward Floating Selection (SFFS) feature selection method to discriminate infected and non-infected segments of leaves of *Arabidopsis thaliana* [27, 28]—a task homologous to species discrimination. Here, we aim at finding the best performing statistical methods that are selected from a broader spectrum of classifiers and feature selection methods. The contrasting features found by this optimized technique are compared with discrimination performance of the conventional ChlF parameters as they are presently used in plant science.

Material and methods

Plant material and growth conditions

Three closely related species: basil (*Ocimum basilicum)*, oregano (*Origanum vulgare*) and sweet marjoram (*Origanum majorana*), all of the family *Lamiaceae*, were selected for the experiment. The seeds of these plants were germinated in pots of 0.08 m diameter containing garden soil at room temperature, under natural relative humidity and light regime at the window of our laboratory. 30–50 plantlets of given species grew in each pot. In another set-up, seeds of the three species were randomly mixed (3×10–20 seeds of each) and germinated in three pots. The ChlF transients were measured 10 days after germination.

Chlorophyll fluorescence imaging

The sequences of ChlF images were measured using open version of FluorCam (Photon Systems Instruments Ltd.), Brno, Czech Republic, [31]. The plant ChlF was excited with short measuring flashes (10–30 μs) and photochemistry was elicited by actinic light from the same set of orange Light Emitting Diodes (LED, 620 nm). The flashes were synchronized with the opening of the electronic shutter of the CCD camera (512×512 pixels, 12 bits) capturing the ChlF images. An optical filter RG695 was placed in front of the CCD camera that blocked the exciting light and allowed to measure the ChlF transient in the range 700–750 nm.

The quenching protocol with two sequentially applied levels of actinic light irradiance was used to measure the ChlF images. This protocol was already used earlier for discrimination between the healthy and infected tissue segments of *Arabidopsis thaliana* and is described in detail in [27, 28]. After measuring sequence of ChlF images, all the pixels of plants were separated from its background using a threshold subtraction. The threshold value was

chosen from the most contrasting image in the sequence. Using this technique, vectors of ChlF transients of all the plant pixels were extracted.

Data analysis tool

The ChlF images were analyzed using Matlab 6.5 and Pattern Recognition Toolbox PRTools v. 3.0 [32]. Algorithms of this toolbox randomly divide the data (vectors of ChlF transients in individual pixels) in two subsets: One subset of transients is used as a reference representing the species (classes) and the other subset is used to perform a simulated classification that serves to assess the algorithm performance. Eight classifiers and four feature selection algorithms were selected from the toolbox to study their efficiency for discrimination of the species.

Classifiers

Statistical classifier is a decision rule that assigns particular ChlF transient to one of the pre-defined classes. We tested the following eight classifiers for their performance and computation time requirement: Linear Discriminant Classifier (LDC) [33–35], Quadratic Discriminant Classifier (QDC) [36, 37], Fisher's Linear Discriminant Classifier (FLDC) [38], *k*-Nearest Neighbor Classifier (*k*-NNC) [39], Nearest Neighbor Classifier (NNC), Automatic Neural Network Classifier (NEURC) [36, 37], Support Vector Classifier (SVC) [40–43] and Nearest Mean Classifier (NMC) [44].

Evaluation of the classifier performance

The ChlF transients recorded in monoculture pots with basil (a), oregano (b), and sweet marjoram (c) plantlets were used for training and evaluating the classifiers performance. The transients were captured from three pots for each species in three consecutive days. Thus, each species was represented by several millions of transients captured in different pixels, representing different leaves and individual plants and taken at different times. Out of this enormous data set, we selected at random ca. 6,600 transients for each species. The thus selected sets of transients P_a, P_b, P_c representing transients of the plant species (a), (b), (c) were randomly and evenly divided into two pixel subsets each: (P_a^{train}, P_a^{test}), (P_b^{train}, P_b^{test}), and (P_c^{train}, P_c^{test}), respectively. The training sets P_a^{train}, P_b^{train}, P_c^{train} were used as a reference to represent the species (a), (b), (c) whereas the mixed testing set ($P_a^{test}+P_b^{test}+P_c^{test}$) was used to evaluate the performance of individual classifiers. Transients arbitrarily chosen from the testing set ($P_a^{test}+P_b^{test}+P_c^{test}$) were compared with each of the training sets (P_a^{train}, P_b^{train}, P_c^{train}) and classified as belonging to one of the three species (a), (b), (c) using the classifier algorithm. The classification was then validated to be either true or false by confronting the classification result with the origin of the particular transient in plant species (a), (b), or (c). The performance of each of the investigated classifiers was quantified by a number between 0–1: value '0' meaning random classification (1/3 of classifications into 3 equally represented classes correct, 2/3 incorrect) and value '1' meaning that the classifier was 100% successful.

Feature selection

The ChlF transients were captured in extensive series of fluorescence images, called features in our study. Feature selection algorithms are designed to reduce the number of features (fluorescence images) for an effective classification [45]. The reduction is based on an identification of an image sub-set that contains the most useful information for plant species identification. Here, we investigated four feature selection methods: Individual Feature Selection (IFS), Sequential Forward Selection (SFS) [46], Sequential Backward Selection (SBS) [46], and Sequential Forward Floating Selection (SFFS) [47].

Table 1 Performance of eight different classifiers (0—random, 1–100% correct) and computation time with 9,900 (9,900/3 for each species) testing and 9,900 (9,900/3 for each species) training transients

Classifier	Performance	Execution time (s)
Linear Discriminant Classifier (LDC)	0.74	14
Linear Discriminant Classifier (LDC)	0.74	14
Quadratic Discriminant Classifier (QDC)	0.82	15
Fisher's Linear Discriminant Classifier (FLDC)	0.75	62
Automatic Neural Networks Classifier (NEURC)	0.77	1,060
k-Nearest Neighbor Classifier (*k*-NN) (*k*=3)	0.76	13,007
Nearest Neighbor Classifier (NN)	0.74	13,793
Support Vector Classifier (SVC)	0.73	28,006
Nearest Mean Classifier (NMC)	0.15	5

For computation we used personal computer with Intel(R) Celeron(R) 2.60 GHz processor and 1.25 GB of RAM.

Results

Classifier performance and execution time

We used eight different algorithms in a simulated classification that was aimed at evaluating their performance and computational time. The results are summarized in Table 1.

With each of the classifiers, we used typically 3,300 testing ChlF transients[1] from each of the three pots with basil (a), oregano (b), and sweet marjoram plantlets (c). These individual testing transients were compared one by one with the training transients of the three species. By that, the algorithms classified each of the testing transients to one of the three species and the classification was counted either as false or correct depending on the true origin of the training transient. With known numbers of correct and false classifications for each of them, the performance of the classifiers was renormalized to 0 if completely random (1/3 of correct classifications in three equally represented classes) and to 1 for 100% of correct classifications. The performance results are shown in the second column of Table 1. Seven out of eight classifiers performed in a narrow range from 0.73 to 0.82.

Table 1 also shows in its third column the computational time required to classify the given number of testing transients. The nearest mean classifier was by far the fastest (5 s) classifier that, however, exhibited the poorest performance. On the other extreme, Quadratic Discriminant Classifier (15 s), and Linear Discriminant Classifier (14 s), and Fisher's Linear Discriminant Classifier (62 s) required low computation time and, yet, exhibited a high performance of species discrimination. Therefore, we choose to continue further evaluation only with Quadratic Discriminant Classifier (QDC) and with Linear Discriminant Classifier (LDC).[2]

Training set size

Another key property of the classifiers affecting their computation time requirement was the size of the training sets that was required for their optimal performance. Figure 1 shows the performance of the two most efficient classifiers, QDC and LDC, increasing with increasing size of the training sets. Above 500 training ChlF transients, the performance of LDC (solid line) started steeply increasing and reached to ca. 0.74 with ca. 3,500 training ChlF transients. A similar but less steep increase in performance

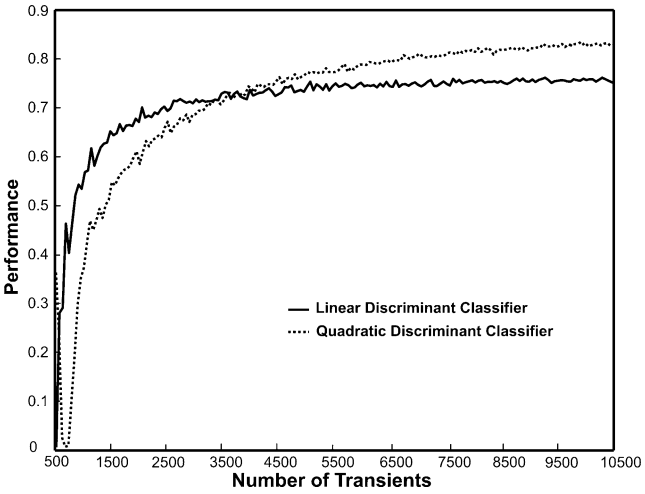

Fig. 1 The performance of the linear (*solid line*) and of the quadratic (*dotted line*) discriminant classifiers as they increase with the number of transients in the training set

was also observed with QDC (dotted line). The QDC classifier kept increasing to ca. 0.82 and outperformed LDC at the training set size larger than 4,500 transients (Fig. 1).

In the classifications documented in Fig. 1 and in Table 1, we used each of the 247 images capturing the entire ChlF transient in our experiments. However, one can possibly assume that some of the images show nearly identical information, such as repeatedly recorded images of F_M or F_0, whereas some other images within the fluorescence transient may be highly contrasting. The most contrasting images can be identified by feature selection algorithms that search for a small subset of features (images) that yield classification performance approaching or even exceeding the performance of the full image sequence. With such a reduced and optimized feature set, one can perform classification more effectively and faster than with the full image sequence.

Comparison of four feature selection methods using the best classifier QDC

Figure 2 shows the performance of QDC when the simulated classification is executed using a reduced number of fluorescence images. Earlier, Tyystjarvi et al. [22] choose to reduce the number of relevant features empirically. Here, we used and compared several feature selection algorithms in an effort to optimize the procedure (Fig. 2).

The reduced set of the ChlF images was identified by four different feature selection algorithms: Individual Feature Selection (IFS), Sequential Forward Selection (SFS), Sequential Backward Selection (SBS) and Sequential Forward Floating Selection (SFFS). The poorest performing algorithm was the Individual Feature Selection (IFS) that evaluated the performance of each single image and choose "the best individual performers" that, under-

[1] Somewhat smaller sets were used with the slower algorithms: with the automatic neural networks classifier (2,250 transients) and with the support vector classifier (1,350 transients) was used for both training and testing set for each species.

[2] The Fisher's Linear Discriminant Classifier is closely related to the Linear Discriminant Classifier.

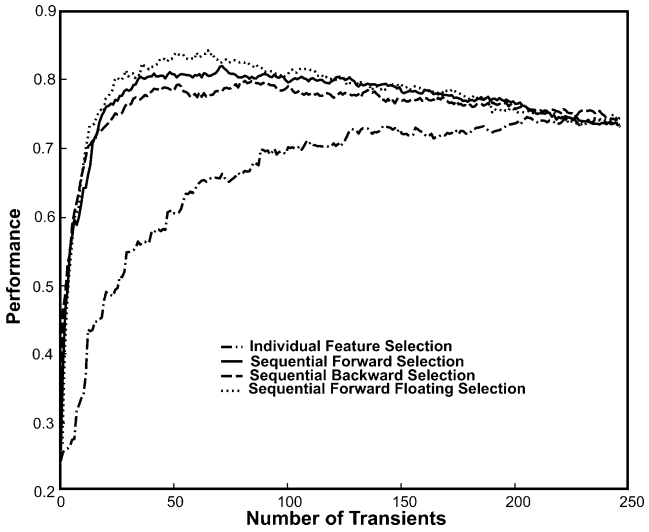

Fig. 2 Performance of the Quadratic Discriminant Classifier, QDC that was applied using a reduced number of fluorescence images that were identified by four algorithms: Individual Feature Selection, Sequentional Forward Selection, Sequentional Backward Selection and Sequentional Forward Floating Selection

standably failed in comparison with "the best performing feature teams". The failure can be understood when considering the model situation in which N images capture the same dynamic feature such as F_0. Assuming that this feature is yielding the highest contrast, these N images will all be selected by IFS in spite of the fact that they contain the same information and the classification performance does not increase with adding more images of the same type. The other feature selection algorithms choose also the best performer but then look for a teammate feature that leads to the highest performance enhancement. This is the principle of the Sequential Forward Selection (SFS). In an alternate way, the algorithm can also work backwards and eliminate from the whole feature set the data that, when not considered in the classification, do not reduce or reduce minimally the overall classification performance. This is the principle of the Sequential Backward Selection (SBS). Slightly better performance was found with training subsets of ChlF images identified by SFS compared to those identified by SBS. The best performing training sub-sets of fluorescence images were found with the SFFS. The highest performance was achieved with approximately a subset of ca. 50–100 images (Fig. 2)[3].

[3] One should note that the feature selection techniques investigated here do not analyze all potential combinations of fluorescence images and in this sense they are called suboptimal. We choose this approach to maintain feasibility with present high performance personal computers. Because of that unexpected time consumption it was impossible to apply optimal techniques such as branch and bound [48], however it would significantly increase the classification performance of the reduced feature sets.

Comparing images obtained with conventional ChlF parameters and with combinatorial imaging

Figure 3 presents the images of three conventional fluorescence parameters: F_0, F_M, and F_V/F_M for a pot in which a random mixture of the three species was grown. The F_0 (Fig. 3a) and F_M (Fig. 3b) signals are largely heterogeneous with slightly higher signals around the center of the image than at its periphery. This effect is probably due to the uncorrected instrument sensitivity profile used in our experiments. Also, the ChlF signals emitted from leaf margins tend to be lower than from the central leaf segments. No obvious dominant classification was observed for individual plants of either species. The F_V/F_M (Fig. 3c) signals are much more homogeneous than F_0 (Fig. 3a) and F_M (Fig. 3b) because the variability due to non-uniform illumination and sensitivity is reduced in ratio. Yet, no dominant species classification was found in Fig. 3c.

In contrast, Fig. 4 represents the classification of individual pixels by the Quadratic Discriminant Classifier, QDC that was used with a sub-set of 50 ChlF images that were identified by the Sequential Forward Floating Selection, SFFS algorithm. Clearly, the top row in Fig. 4 shows that the classification was successful when applied on single species pots.[4] The Fig. 4a shows a pot with basil (*Ocimum basilicum*, L.) that was mostly classified correctly (red color) with only few pixels classified erroneously by green color as oregano (*Origanum vulgare*, L.) and by blue color as majoram (*Origanum majorana*, L.). The pot in Fig. 4b with oregano was also mostly classified correctly (green color) with only few pixels classified erroneously by red color as sweet basil and few by blue color as marjoram. Similarly, the classification of marjoram in Fig. 4c was dominantly correct (blue color).

Figure 4d shows classification result in a more realistic situation where all the species were mixed in a single pot. In contrast to the conventional ChlF parameters shown in Fig. 3, the combinatorial imaging shown in Fig. 4d leads to a clear contrast with red pixels classified as basil (large leaves) contrasting with the two other two species: blue pixels classified as sweet marjoram, and green pixels as oregano.

Discussion

The simplest and most intuitive among the classifiers tested is the nearest mean classifier, NMC, which is representing each species by a single transient that is obtained by

[4] These pots were different from those used for classifier training.

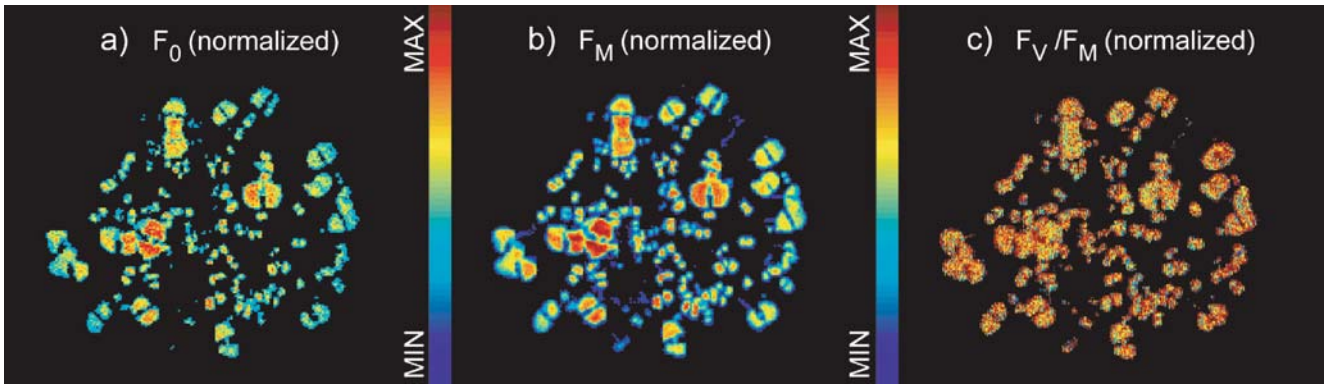

Fig. 3 The images **a**, **b**, and **c** represent the conventional fluorescence parameters F_0, F_M and F_V/F_M of a pot with randomly mixed specie. The fluorescence parameters are shown using a rainbow false color scale with minimum values shown in blue and maximum values shown in red. Normalization was done by subtracting the minimum value and further division by the difference between maximum minus minimum value, i.e. $\frac{F-\min(F)}{\max(F)-\min(F)}$

averaging all transients in the respective training class [44]. Unknown transients are then compared with each of the averaged representative transients of the individual species and classified to the species that is the most similar. The performance of this classifier was found in our experiments to be very low around 0.15 (Table 1). The NMC classifier failed because it does not reflect width of the statistical distribution in the training classes.

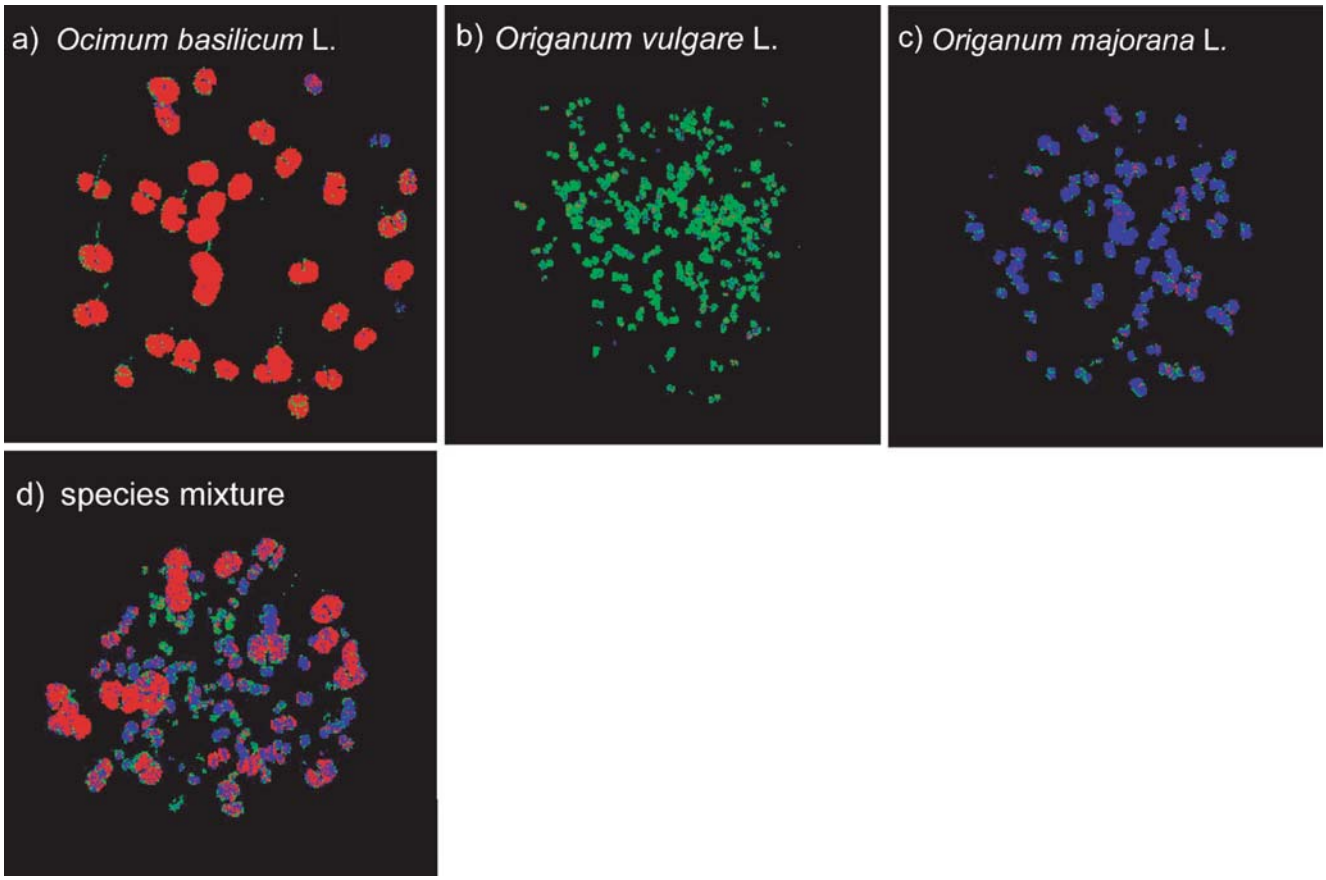

Fig. 4 The panels **a**, **b**, and **c** show QDC classification of fluorescence transients in individual pixels of images with pots containing seedlings of basil (*Ocimum basilicum*, L.), oregano (*Origanum vulgare*, L.) and marjoram (*Origanum majorana*, L.). The pixels classified as sweet basil are shown in *red*, those classified as oregano are shown in *green* and the pixels classified as marjoram are shown in *blue*. The same color code of classification is used in panel **d** that shows a pot in which all the three species grew mixed together

The other seven classifiers were of similarly high performance (0.73 to 0.82). Among these, we selected the best classifier based on its computation time. The computation time of the Support Vector Classifier (SVC) was the longest (ca. 8 h with a standard PC, Table 1). The long computation time of SVC was required for transformation of the original data into a space of a higher dimension that was followed by finding a linear separating surface between the two classes [49]. The *k*-Nearest Neighbor Classifier (*k*-NN), the Nearest Neighbor Classifier (NN) and the Automatic Neural Networks Classifier (NEURC) were also requiring excessive computation time. The optimal combination of high performance with acceptable computation time was found with the Quadratic Discriminant Classifier (QDC) (performance 0.82 in 15 s of computation time). The Linear Discriminant Classifier (LDC) achieved the second best classification performance of ca. 0.74 with 14 s of the computation time. The LDC and QDC identify a linear and quadratic hyperplane, respectively, that best separates the training transients of the individual species. The classification consists in finding on which side of the hyperplane the classified transient is. The classification performance of QDC (0.82) was better than that of the LDC (0.74) because the training classes were of different distribution leading to different covariance matrices and, eventually, to non-linear separating hyperplane.

With QDC and LDC identified as the two best performing classifiers, one needs to optimize the size of the training sets [50]. This was done by investigating the classification performance with increasing size of training data sets (Fig. 1). With very small training data sets the performance was not stable as the number of training transients was not sufficient to reliably place the classification hyperplane. The performance of LDC rapidly increased with increasing the number of training transients to 3,500. Above 3,500 training transients the position of the linear hyperplane was near to optimum and addition of training transients to 4,500 and more led to no improvement of performance. Optimal placing of a quadratic hyperplane that separates the transients of two species is harder and requires more training data. Thus, the performance of QDC was increasing less steeply compared to LDC and outperformed LDC only with ca. 4,500 training transients. In contrast to LDC, the QDC performance continued increasing above 8,100 training ChlF transients where we choose to limit our search because of computation time limits.

With QDC as the best classifier, we further optimized the species discrimination by feature selection. Both SFS and SBS are more effective than IFS (Fig. 2). The best results were obtained with the Sequential Forward Floating Selection (SFFS). The SFFS works in the similar way as SFS algorithm, but after every addition of an image, it checks if removing another image from the selection would not lead to a better performance than one step back. By checking such potential "backward steps", the SFFS algorithm identified feature sets that were performing slightly better than the sets found by SFS or SBS (Fig. 2). In our experiments, the SFFS algorithm reduced the full data set of 247 images to 50 images that were the most effective. The classification with 50 images was significantly faster than with the full data set (247 images) without compromising the classification performance.

Our results show that the Quadratic Discriminant Classifier (QDC) utilizing only 50 images found by the Sequential Forward Floating Selection (SFFS) constitutes a robust and effective method for plant species discrimination based on ChlF transients. In our simulated species discrimination, the conventional ChlF parameters such as F_0, F_M, and F_V/F_M shown in Fig. 3 as well as other conventional parameters that were tested but not shown (NPQ, qP, Rfd) failed. The low discriminative potential of these parameters is understandable because the closely related plants have similar chlorophyll density in leaves, similar leaf structure and similar photosynthetic yield [40, 51]. The natural variability of the ChlF emission and low contrast between the closely related species do not allow effective species discrimination.

In contrast, the combinatorial species discrimination represented in Fig. 4 was highly effective. When tested with a pot in which all three species were mixed (Fig. 4d) the contrast between the species was preserved although some erroneously classified pixels were detected, probably, because of leaf overlaps that were prominent in the two small leaf species (oregano and marjoram). The classification of basil with large leaves was typically correct even in the pot with mixed species (basil).

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