Camellia japonica L. genotypes identified by an artificial neural network based on phyllometric and fractal parameters

S. Mugnai, C. Pandolfi, E. Azzarello, E. Masi, S. Mancuso

Department of Horticulture, University of Florence, Florence, Italy

Abstract. The potential application of phyllometric and fractal parameters for the objective quantitative description of leaf morphology, combined with the use of Back Propagation Neural Network (BPNN) for data modelling, was evaluated to characterize and identify 25 *Camellia japonica* L. accessions from an Italian historical collection. Results show that the construction of a BPNN based on phyllometric and fractal analysis could be effectively and successfully used to discriminate *Camellia japonica* genotypes using simple dedicated instruments, such as a personal computer and an easily available optical scanner.

Keywords: backpropagation neural network (BPNN); *Camellia*; cluster analysis; cultivar identification; fractal spectrum

Introduction

The need to preserve genetic variability has been realized in crop field since a long time through the development of germplasm conservation programmes and the establishment of gene banks. On the contrary, the safeguard of varietal variability has been only recently undertaken on ornamental species (Petrova 1996). Genetic erosion assumes an alarming significance especially in those species in which genetic improvement has originated an extremely high number of cultivars, with a consequent loss or oversight of the ancient ancestors. Among these, *Camellia japonica* L. (Theaceae) represents a bright example, totalling currently about 30,000 cultivars. Camellia cultivation has a long history and the wide range of flower forms (e.g. single, anemone, formal), colours, and sizes is the result of many centuries of selection for desirable characteristics, first in China and Japan (Durrant 1982, Chang and Bartholomew 1984), then in Europe. The introduction of *Camellia japonica* L. in Italy is dated about 1760 (Remotti 2002), but only during the XIXth century this species reached a high productive importance, with the selection of brand new cultivars (Corneo et al. 2000). In particular, Florence and Lucca became important growing areas, due both to their favourable ecological conditions and the work of some breeders and collectors such as Oscar Borrini and Filippo Parlatore (Grilli 1881, 1883). Nowadays, the Italian production, even if it covers a considerable economic importance, is limited to the commercialization of about 200 cultivars, mostly derived

Correspondence: Sergio Mugnai, Department of Horticulture, University of Florence, Viale delle Idee 30, 50019 Sesto Fiorentino, Italy
e-mail: sergio.mugnai@unifi.it

from Eastern Asian ones. A worrying phenomenon that involves Italian old *Camellia japonica* cultivars is the loss of identity, due to frequent cases of synonymy, homonymy and wrong naming (Remotti 2002), so the need of restoring the correct names by the use of genetic and/or morphological traits. Traditional diagnostic keys for naming *taxa* based on morphological studies have long played a fundamental role with regard to practical biological identification. Bracketed or indented keys, dichotomous or otherwise have the advantage that they can easily be edited in a printed page, but have also some disadvantages, including a high level of diagnostic skill and the knowledge of specialised terminology needed (Clark and Warwick 1998). These traditional morphological methods for characterization and assessment of genetic variability are time consuming, often affected by the environment and can be only easily used to distinguish between different species. On the contrary, problems can arise at variety and clone levels because the previous methods are mainly based on subjective visual assessment, often unable to detect small differences. Interesting perspectives have been highlighted in cultivar discrimination by the analysis of isoenzymes (Sánchez-Escribano et al. 1999), chemical compounds of phenolic nature having taxonomic value (Eder et al. 1994) and nucleic acids, mostly DNA. Simple sequence repeat polymorphism (SSRP), randomly amplified polymorphic DNA (RAPD) (Zebrowska and Tyrka 2003), inverse sequence-tagged repeat (ISTR), microsatellite variability (Sefc et al. 2000), amplified fragment length polymorphism (AFLP) have proven to be useful tools for characterization of ornamental varieties (Lombard et al. 2001). For example, in *Camellia japonica* L. the genetic structure of a wild population was investigated using microsatellite markers (Ueno et al. 2000, 2002). Moreover, there are phylogenetic studies conducted in order to understand the relationships inside the family of Theaceae using chloroplast DNA sequence data (Prince and Parks 2001) or isoenzymes (Parks et al. 1995), but none of them had the aim and/or the capacity to discriminate between cultivars of the same species. These biomolecular techniques, though effective, are resource and labour-intensive, and require a skilled and experienced technical staff to be effectively exploited. A new exciting perspective in plant identification has been recently developed from a modern and powerful technique: the use of artificial neural networks, or ANNs. An ANN is an information processing paradigm structured as biological nervous systems, such as the brain, composed of a large number of highly interconnected processing elements (like neurons) working in unison to solve specific problems. ANNs, like the human mind, learn by examples. An ANN is configured for a specific application, such as pattern recognition or data classification, through a learning process. The possibility of using artificial neural networks based on morphological traits for plant identification has been tested a few years ago (Clark and Warwick 1998, Mancuso and Nicese 1999, Mancuso et al. 1999, Clark 2004), while their use in other areas of science and technology have advanced knowledge (i.e. voice and handwriting recognition, vibration analysis, diagnostic in medicine, elemental particle identification in physics). The most useful qualities of ANNs, such as their skill and speed in recognizing pattern and shapes (Hertz et al. 1991), have barely been exploited in ornamental plants (Pandolfi et al. 2006).

The aim of this research is the morphological characterization of ancient *Camellia* cultivars from a historical Italian collection (Villa Orsi, near Lucca) by using quantitative morphological (i.e. phyllometric) and fractal spectra traits originated from leaves image analysis, and their discrimination by building a specific ANN for data modelling.

Materials and methods

Plant material. Twenty-two old *Camellia japonica* L. cultivars were selected in the historical garden of Villa Orsi in Compito (Lucca, Italy). The complete list of the accessions is showed in Table 1. From each accession fully expanded and healthy leaves were randomly collected in late spring according to uniformity of appearance, growth habit and exposure. The samples were chosen excluding the non-representative and anomalous plants.

Table 1. List of *Camellia japonica* accessions from Villa Orsi (Compito, Italy)

Camellia japonica accessions from Villa Orsi Garden			
1	Alba Plena	12	Oscar Borrini
2	Bonardi	13	Paolina Maggi
3	Chandleri	14	Principessa Baciocchi
4	Drouard Guillon	15	Prof. Giovanni Santarelli
5	Giardino Santarelli	16	Punicaeflora
6	Giovanni Nencini	17	Roma Risorta
7	Ignea	18	Rubina
8	Il Gioiello	19	Rubra Simplex
9	Lavinia Maggi Rubra	20	Sacco Vera
10	Madame Pepin	21	San Dimas
11	Marmorata	22	Violacea Superba

Image acquisition and determination of phyllometric parameters. An optical scanner (CanoScan D660U), set at 300×300 dpi and 16 million colours, was used to acquire leaf images (Fig. 1). Fourteen phyllometric parameters (Table 2) were determined for each leaf image, previously transformed in a 256 grey scale, using an image analysis software (UTHSCSA Image Tool 3.0, freeware at ddsdx.uthscsa.edu/dig/itdesc.html).

Fractal dimension and fractal spectrum. Fractal parameters were determined through a fractal image analysis software (HarFA, Harmonic and Fractal Image Analyzer 4.9.1, freeware at www.fch.vutbr.cz/lectures/imagesci). The leaf fractal spectrum was obtained using the method previously described by Mancuso (2002). Briefly, each leaf colour image was split in three constituting colour channels (red, green and blue); each channel was thresholded for a colour value between 0 and 255 and the fractal dimension for each colour value was then assessed using the box-counting method. The implementation of these methods has been described in details by Mancuso et al. (1999b). After drawing the baseline (fractal dimension = 1) which separates the fractal (>1) from the non-fractal (<1) zone of the spectrum, five fractal parameters (First X, Peak X, Last X, Peak Y and Total Peak Area) for each channel were calculated (Fig. 2). Figure 3 shows an example for the spectra of the three colour channels (blue, green and red). According to Mancuso et al. (2003), the green and red channels were influenced by the phenotype, shifting their respective spectra to the left or to the right according to the different accessions, whereas the blue channel seemed relatively unaffected. As a consequence, only the green and the red channels were selected as informative for the constitution of the neural network.

Construction of an ANN. The most suitable type of network for plant identification is considered to be the supervised backpropagation neural network (Mancuso and Nicese 1999), which is a particular kind of multilayer feed-forward network, or multilayer perceptron (MLP). Briefly, a BPNN has a layered structure, with its architectural layout basically composed by some layers of neurons, namely the input layer, one or more hidden layers and the output layer. Each layer receives its input from the previous layer or from the network input, whereas the output of each neuron feeds the next layer or the output of the network. Particular nodes were also used to shift the neuron transfer function and to improve the network performance, thanks to the backpropagation of errors. As the backpropagation of errors is well described in literature (Rumelhart et al. 1986, Haykin 1999), only the final equations used in this study for the correction of weights are provided. The most frequently used adjustment of weights is a simple gradient descent: in this case, each weight adjusts by a small amount proportional to the derivative of the error function ($\delta E / \delta w_{ij}$) with respect to that weight, and in the opposite direction:

$$\Delta w_{ij} = -\varepsilon \ (\delta E / \delta w_{ij}) \tag{1}$$

where Δw_{ij} is the adjustment of the weight w_{ij} connecting the unit i of one layer with the unit j of the following layer, while ε is the learning rate usually variable in the range between 0.001 and 1.0.

In the present study a strict gradient descent (Rumelhart and McClelland 1988) was used with the introduction of an averaging term:

$$\Delta w_{ij} \ (\text{iteration } n) = -\varepsilon \ (\delta E / \delta w_{ij}) \\ + \mu \Delta w_{ij} \ (\text{iteration } n - 1) \tag{2}$$

where the coefficient μ is referred as the momentum. The RMS (root mean square) error between the network response ($X_{(P,K)}$) and the training targets ($T_{(P,K)}$) was computed after each iteration according to the equation:

$$\text{RMS error} = \text{SQRT}(\Sigma_{P,K}((T_{(P,K)} - X_{(P,K)})^2)/(P_T \cdot K_T)) \tag{3}$$

where P is the Pth input pattern and K is the Kth output node. P_T is the total number of patterns, and K_T is the total number of output nodes.
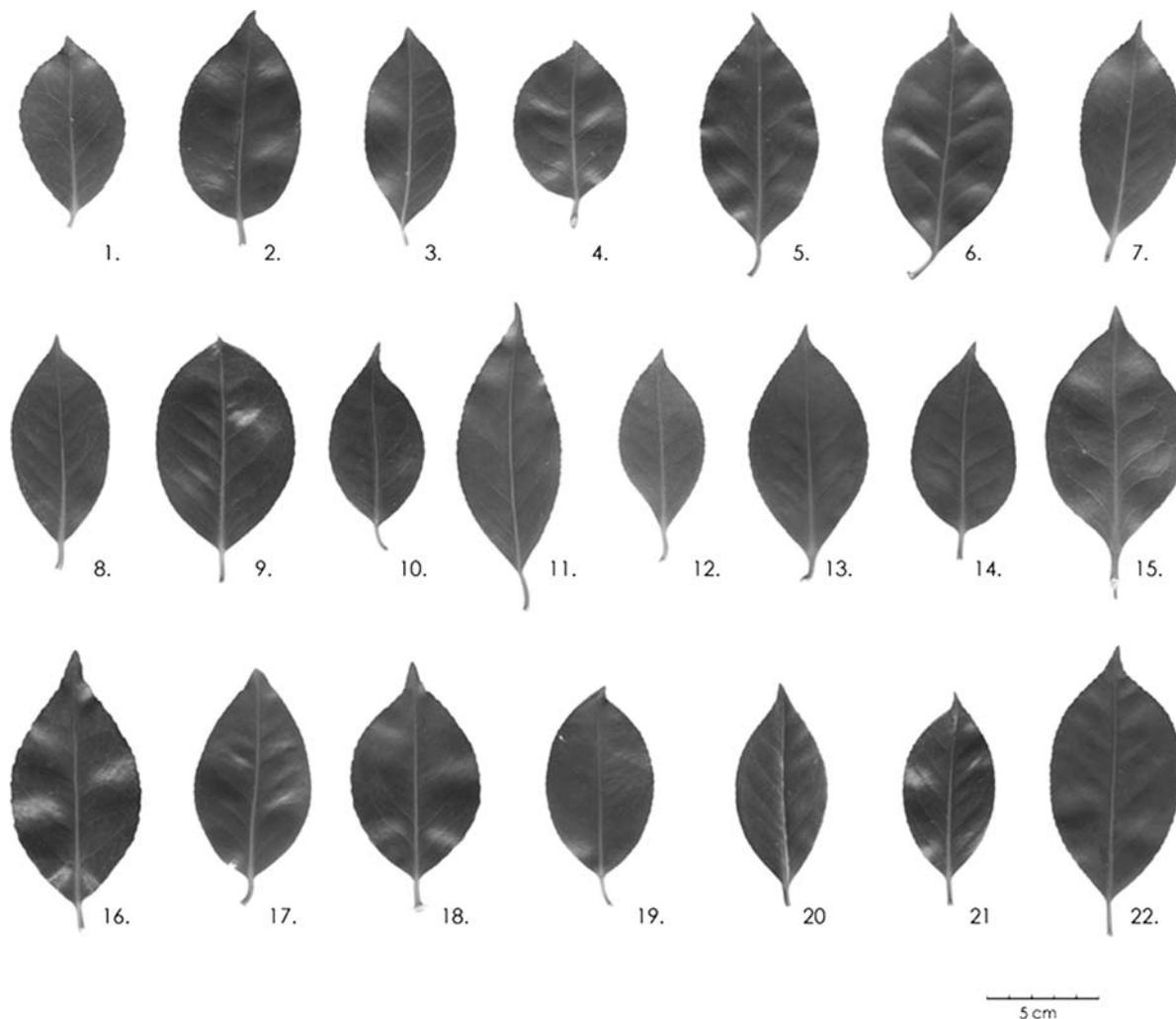
Fig. 1. Leaves images: **1** Alba plena, **2** Bonardi, **3** Chandleri, **4** Drouard Guillon, **5** Giardino Santarelli, **6** Giovanni Nencini, **7** Ignea, **8** Il Gioiello, **9** Lavinia Maggi rubra, **10** Madame Pepin, **11** Marmorata, **12** Rubina, **13** Oscar Borrini, **14** Paolina Maggi, **15** Principessa Baciocchi, **16** Prof. Giovanni Santarelli, **17** Punicaeflora, **18** Roma Risorta, **19** Rubra Simplex, **20** Sacco Vera, **21** San Dimas, **22** Violacea Superba

The network was designed using a total of 24 inputs signals (learning phase) represented by the phyllometric and leaf fractal parameters, and 22 outputs represented by the *Camellia* accessions (recognition phase). In total, data from 1100 leaves (50 per each cultivar) were used. From preliminary tests, 50 was considered the minimum significant number of leaves per each phase. To optimize the neural network activity, the number of hidden neurons and the number of iterations was modified. Concerning the hidden layer, many factors such as learning scheme, number of nodes of the output and the input, and connections between them play an important role for the determination of the best configuration (Zurada and Malinowski 1994). In our case, minimum error was reached with a network composed of 50 hidden neurons, positioned on one level, with the hidden layer activated by a logistic sigmoid activation function:

$$y = \frac{1}{(1 + e^{-x})} \qquad (4)$$

The learning phase was protracted until the RMS error became less than 0.06, and the difference between the RMS in two consecutive steps was <0.0001.
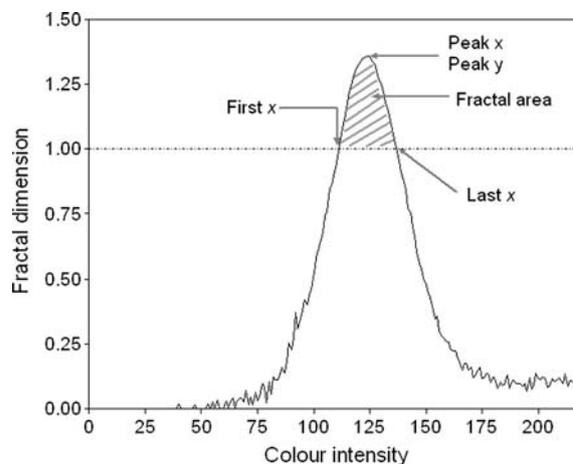
Table 2. Morphological parameters inputs measured by the image analysis software

	Parameter	Definition
1	Area	The area of the leaf
2	Perimeter	The perimeter of the leaf
3	Major axis length	The length of the longest line that can be drawn trough the leaf
4	Minor axis length	The length of the longest line that can be drawn trough the leaf perpendicular to the major axis
5	Roundness	Computed as: $(4 \times \pi \times area) / perimeter^2$
6	Elongation	The ratio of the length of the major axis to the length of the minor axis
7	Feret diameter	The diameter of a circle having the same area of the leaf
8	Compactness	Computed as: $sqrt(4 \times area / \pi) / major axis length$
9	Integrated density	Computed as the product of the mean grey level and the number of pixels in the image of the leaf
10	Min grey level	Minimum grey level of the leaf
11	Mean grey level	Mean grey level of the leaf
12	Median grey level	Median grey level of the leaf
13	Mode grey level	Mode grey level of the leaf
14	Max grey level	Maximum grey level of the leaf

Fig. 2. Graphical representation of the five fractal parameters calculated from each colour channel: first X, peak X, last X, peak Y and total peak area

Neural network outputs of the BPNN were used to measure the dissimilarities or distances between ecotypes when forming the clusters. Euclidean distances were calculated and a dendrogram was constructed based on the distance matrix data by applying an unweighted pair group method with arithmetic averages (UPGMA) cluster analysis.

Results

Learning and recognition phase. Two separate groups of leaves from the whole collection were preliminary created, one for the learning phase, the latter for the prediction one, in order to test the network capacity to create effective rules for the characterization of each accession. The ANN output by phyllometric and fractal parameters inputs has been represented by a XY-graph for each accessions, with the cultivar names on the x-axis, whereas the y-axis is the output for the level of similarity obtained comparing the leaves from the learning phase and the leaves from the recognition one. Each graph aims to show how the ANN was able to discriminate the selected accession in comparison with the others. The level of similarity is expressed by a number that ranges between 0 (false) and 1 (true). In an ideal case, only one output class has the averaged output signal equal to 1, while all the other outputs have their value equal to 0. Obviously, this is a theoretical result. Due to the natural variation among leaves, the output of the expected class tends to report a value close to 1, but less than 1, while the others should be close to 0. Three different situations were observed in the tested *Camellia* genotypes: a) *complete recognition*, when the output graph shows one evident peak in the expected class; b) *failed recognition*, when the expected class peak value is very low; c) *partial recognition*, when the expected class evident peak was also associated to other almost equal peaks, indicating a close morphological similarity among two or more accessions.
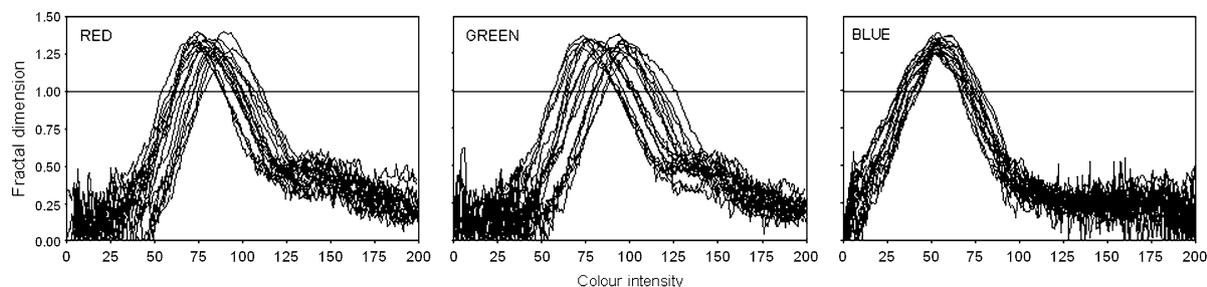
Fig. 3.   Fractal spectra of the red, green and blue channels of a single leaf for each camelia accession

A clear example of *complete recognition* is shown in Fig. 4, where 'Alba Plena' and 'Drouard Guillon' genotypes reached the highest output only in the expected class, with averaged values around 0.45 and 0.6, respectively, whereas the other classes had negligible output values. 'Bonardi', 'Giardino Santarelli', 'Giovanni Nencini', 'Ignea', 'Lavinia Maggi Rubra', 'Marmorata', 'Oscar Borrini', 'Paolina Maggi', 'Principessa Baciocchi', 'Prof. Giovanni Santarelli', 'Rubra Simplex', 'Sacco Vera' and 'San Dimas' cultivars were also completely and univocally recognized by the network, as no evident peaks of similarity with other tested cultivars were noticed (data not reported). On the contrary, Fig. 5 shows a failed recognition example: the very low output values of the expected classes for 'Il Gioiello' and 'Madame Pepin' were not suitable for an effective genotype identification (*failed recognition*). In other cases, the ANN was partially able to discriminate among two accessions (*partial recognition*), without an univocal response. For example, the expected class for 'Chandleri' had the same output value of an unexpected class, 'Rubina', and vice versa (Fig. 6). This result is probably derived from a morphological similarity between the two accessions that may correspond to a genotypic similarity. Other partial recognitions were detected for 'Roma risorta' (confounded with 'Lavinia Maggi Rubra') and 'Violacea Superba' (confounded with 'Giardino Santarelli). In the last two cases, no inverse partial recognition was detected, as noticed for the previous two cultivars. Resuming the results, a successful identification was observed in 15 classes, a partial

identification in 4 classes, while no identification in 3 classes (Table 3).

Prediction phase. Ten leaves from the same accessions were processed later as previously described and introduced to the network as 'unknown leaves' in order to check the validity of our network at a different time since its creation. The introduction of data from some unknown leaves to the network was used to onset a newly created output in order to match the similarity between the unknown leaves and the corresponding leaves used for the learning phase. As expected, the BPNN was able to associate the 'unknown leaves' to the correspondent genotypes, except for a few cases. For example 'Drouard Guillon' reached the highest output only in the expected class, with the averaged value around 0.45, whereas the other classes had negligible output ones (Fig. 7a) while the network was not able to clearly and completely identify the unknown leaves of 'Principessa Baciocchi' (Fig. 7b), even though the cultivar was completely and univocally recognized in the previous recognition phase. The graph shows some small but not significant peaks, distributed among five accessions, such as 'Alba Plena', 'Drouard Guillon', 'Ignea', 'Principessa Baciocchi' and 'Rubina'. In this case the network was not able to correctly collocate the group of leaves collected in a different period of the year: probably, the unknown leaves were significantly different from those used in the training phase. Figure 8 shows the prediction test result on 'Chandleri' genotype previously described in Fig. 6: again, the network associated to 'Rubina' some unknown leaves
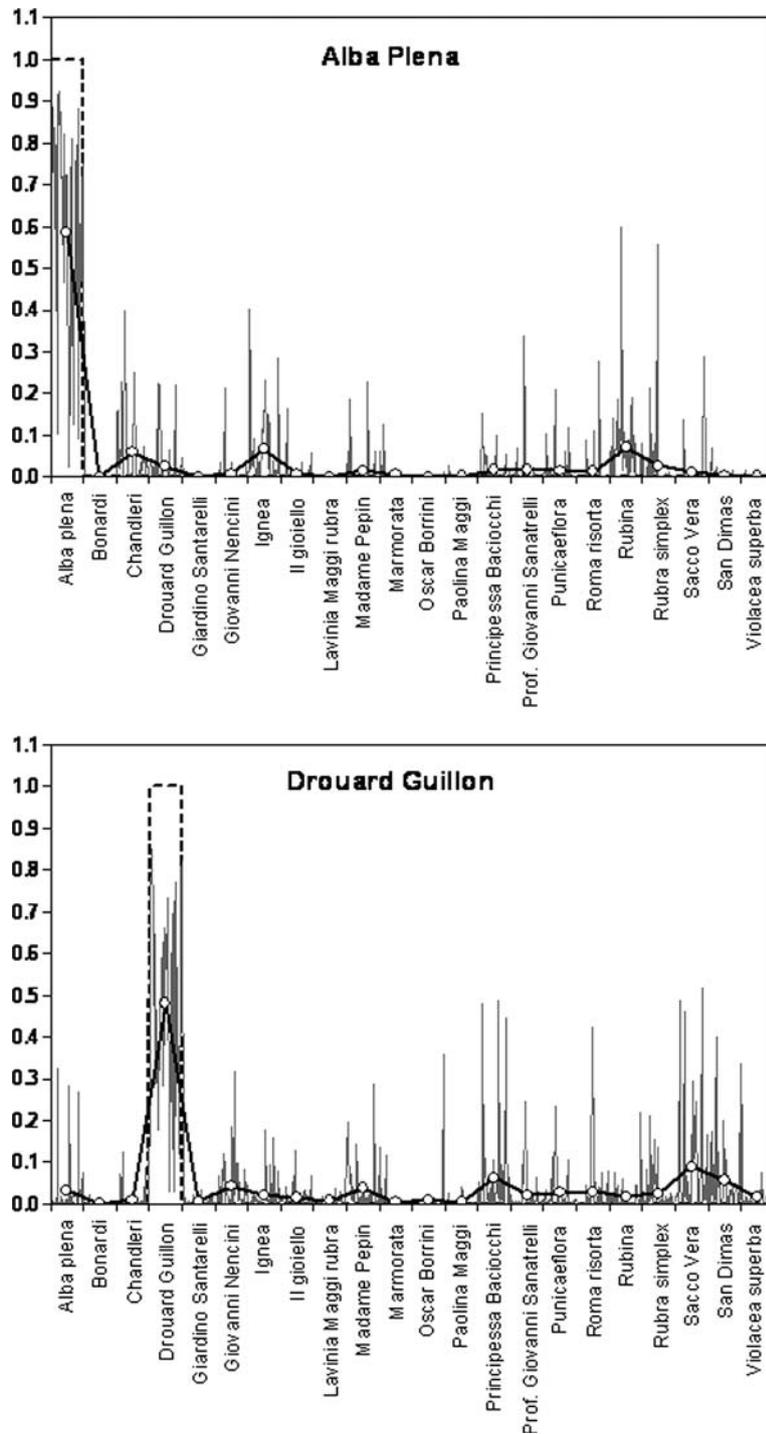
Fig. 4. Examples of a complete recognition by the BPNN. Each frame shows the BPNN output for the input represented by the phyllometric and fractal parameters of 40 leaves. Reported lines show the averaged output data
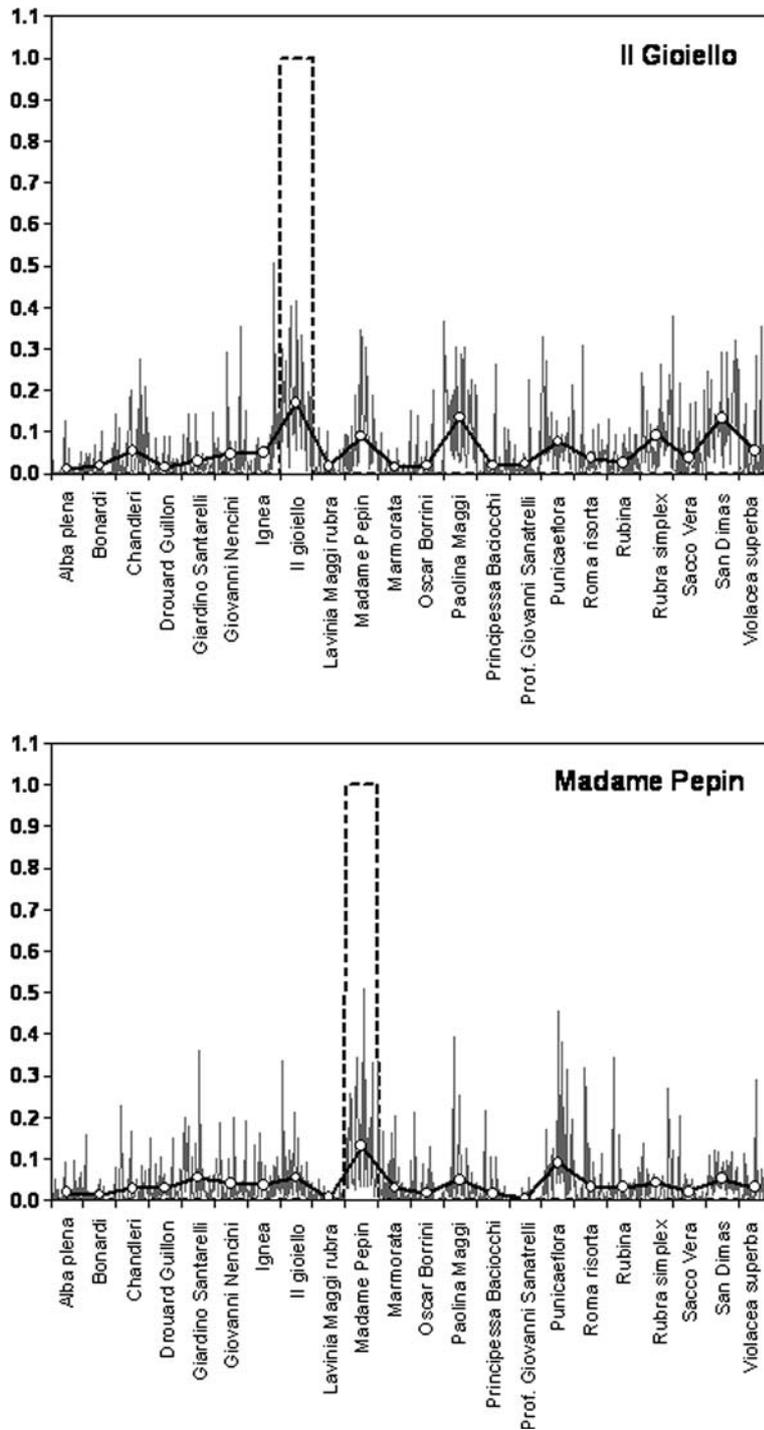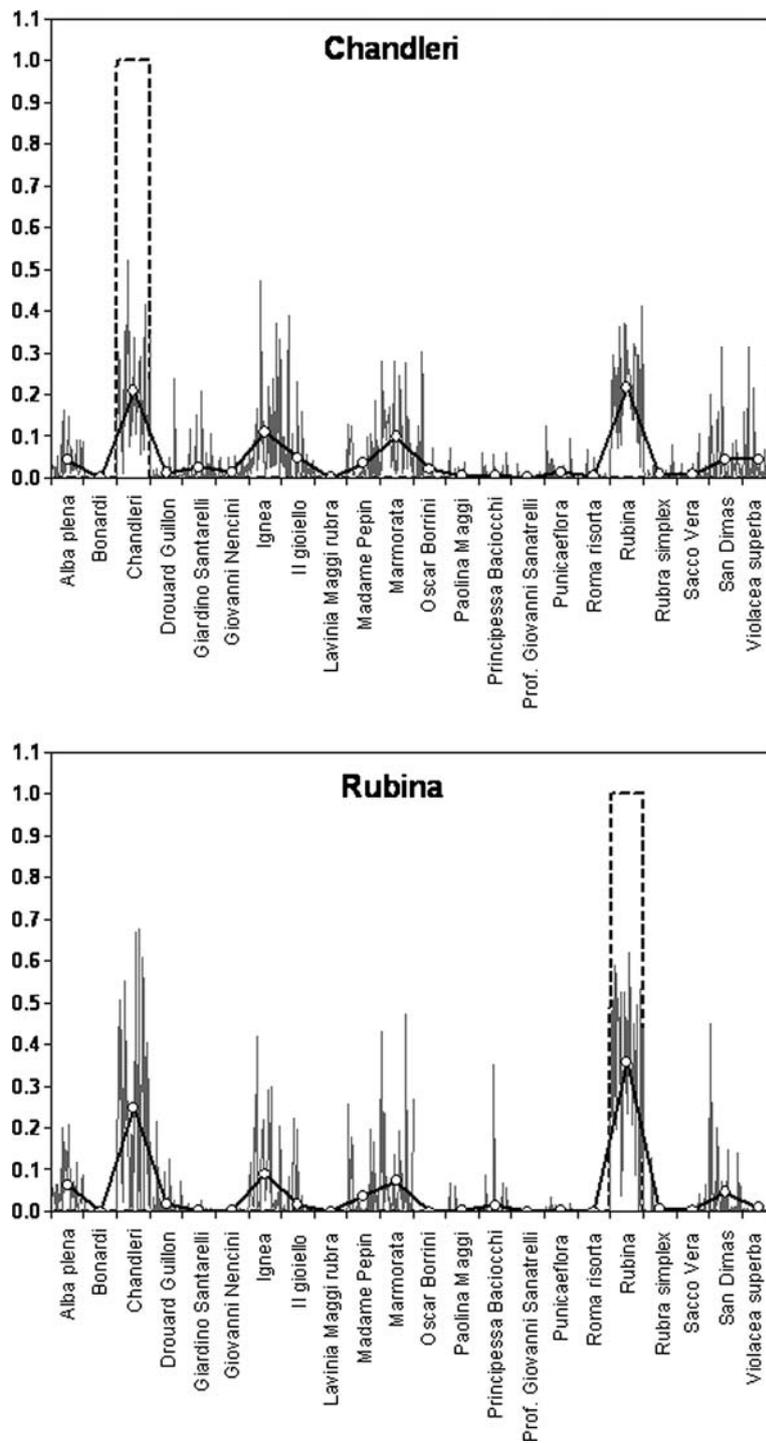
Fig. 5. Examples of a failed recognition by the BPNN. Each frame shows the BPNN output for the input represented by the phyllometric and fractal parameters of 40 leaves. Reported lines show the averaged output data

Fig. 6. Examples of a partial recognition by the BPNN. Each frame shows the BPNN output for the input represented by the phyllometric and fractal parameters of 40 leaves. Reported lines show the averaged output data

Table 3. Total, partial, or failed recognitions obtained by the BPNN

Complete recognition	Partial recognition
Alba Plena	Chandleri
Bonardi	Rubina
Drouard Guillon	Roma Risorta
Giardino Santarelli	Violacea Superba
Giovanni Nencini	
Ignea	
Lavinia Maggi Rubra	
Marmorata	**Failed recognition**
Oscar Borrini	
Paolina Maggi	Il Gioiello
Principessa Baciocchi	Madame Pepin
Prof. Giovanni Santarelli	Punicaeflora
Rubra Simplex	
Sacco Vera	
San Dimas	

collected from 'Chandleri', reiterating the previous error.

Cluster analysis. The BPNN outputs have been analyzed using cluster analysis to deeply understand the similarities among the tested phenotypes. This kind of analysis is a useful tool for grouping objects of similar kind into respective categories, as all the similarities and dissimilarities are evaluated, in a way that the degree of association between two objects is maximal if they belong to the same group and minimal otherwise. The UPGMA cluster analysis of the distance matrix (Fig. 9) separated the *Camellia japonica* accessions into different groups, plus one isolated accession ('Alba Plena'). The clusters represent the groups of similar accessions. For example, 'Lavinia Maggi Rubra' together with 'Roma Risorta' and 'Il Gioiello' together with 'Madame Pepin' had very close connections, showing their high level of similarity and the possibility to have a strong genetic linkage. More, 'Chandleri', 'Rubina', 'Ignea' and 'Marmorata' are all grouped in the same cluster due to their morphological characteristics confirming the previously described neural network results.

Discussion

Results herein reported demonstrate that a back-propagation neural network (BPNN) can be used to effectively differentiate *Camellia japonica* L. cultivars through phyllometric and fractal parameters, as the majority of the tested genotypes were univocally discriminated during the recognition phase. Similar positive results have been previously obtained in olive by Mancuso and Nicese (1999), in chestnut by Mancuso et al. (1999) and in grapevine by Mancuso (1999a, 2002). Much of the botanical identification is still carried out using taxonomic keys, which is a classical paper-based kind of expert system. The success and accuracy of this identification relies heavily on the experience and the interpretation of the user, as traditional discrimination methods based on morphological studies are mainly based on subjective visual assessment, which is often unable to detect small differences as those among cultivars. In our case, the cultivar discrimination of *Camellia japonica* L. based on these methods such as leaf attributes determination can not lead to satisfactory results as most of the tested genotypes show very similar leaf dimensions and shapes (Fig. 1). On the contrary, the building of an artificial neural network based on morphometric and fractal leaf parameters successfully led to an effective genotype recognition, even though particular care must be directed to the choice of the leaves, which must be healthy and well-developed. For example, the probable reason to explain the no-recognition situations was that the high variability in the leaf shape inside these cultivars did not permit the creation of certain rules for their univocal characterization. The study of the fractal spectrum has been recently used as useful tool to assess the hardiness and cold tolerance of ornamental plants such as *Callistemon* and *Grevillea* spp. (Mancuso et al. 2003, 2004) but, to our knowledge, this is the first attempt to successfully introduce leaf fractal parameters as a botanical identification key to be inserted in an artificial neural network at the input layer. Our BPNN is also a powerful tool in order to detect close similarities among the genotypes. The case of 'Chandleri' and
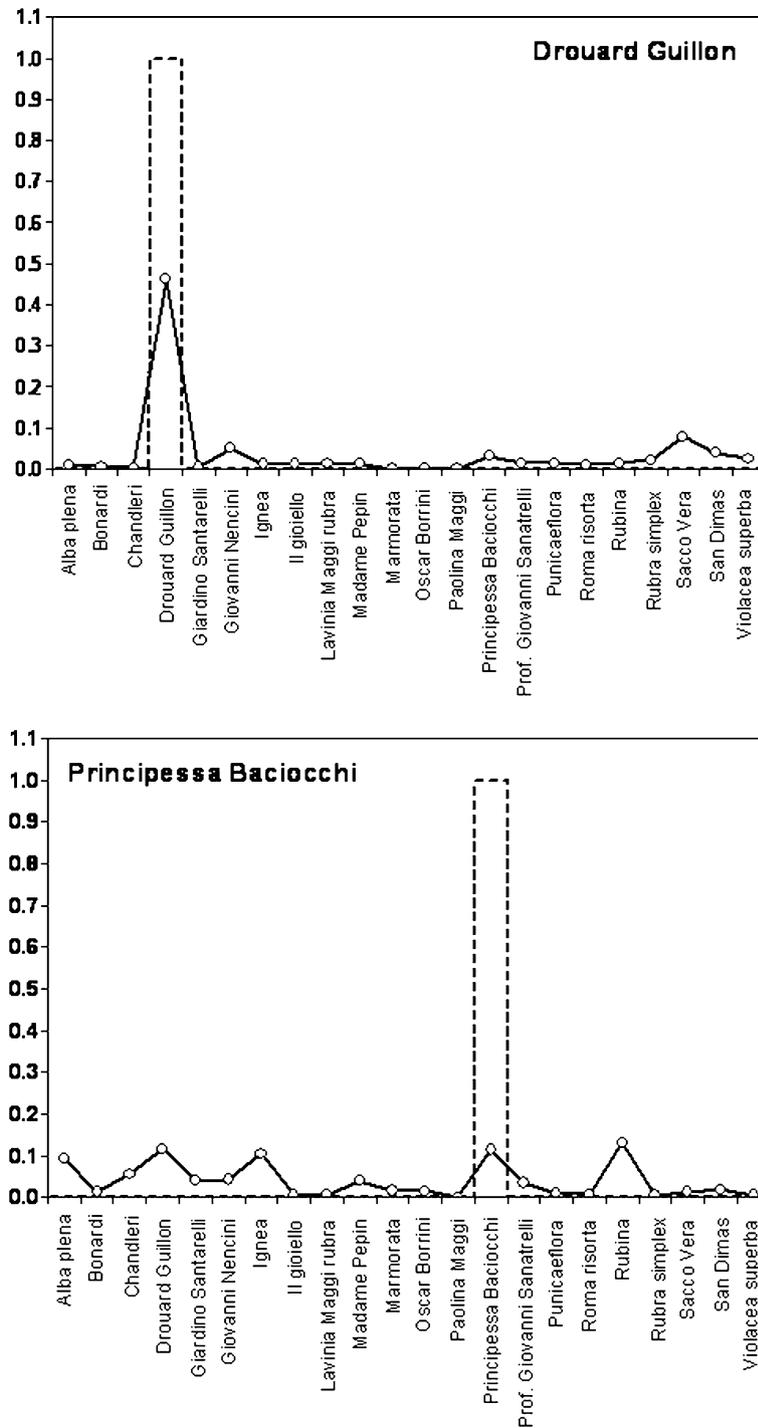
Fig. 7. Prediction phase output for 'Drouard Guillon' and 'Principessa Baciocchi'

'Rubina' is a good example (Fig. 6). The two accessions seem to be phenotypically very close, with a likely common ancestor: in fact, looking to their flower characteristics, both have Anemone-form red flowers even though their selection was conducted in two different locations.
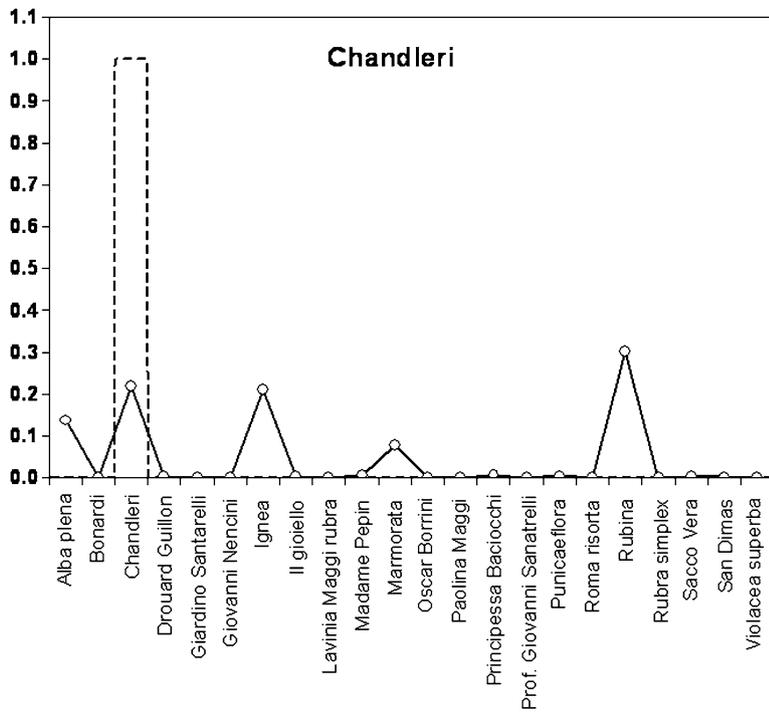
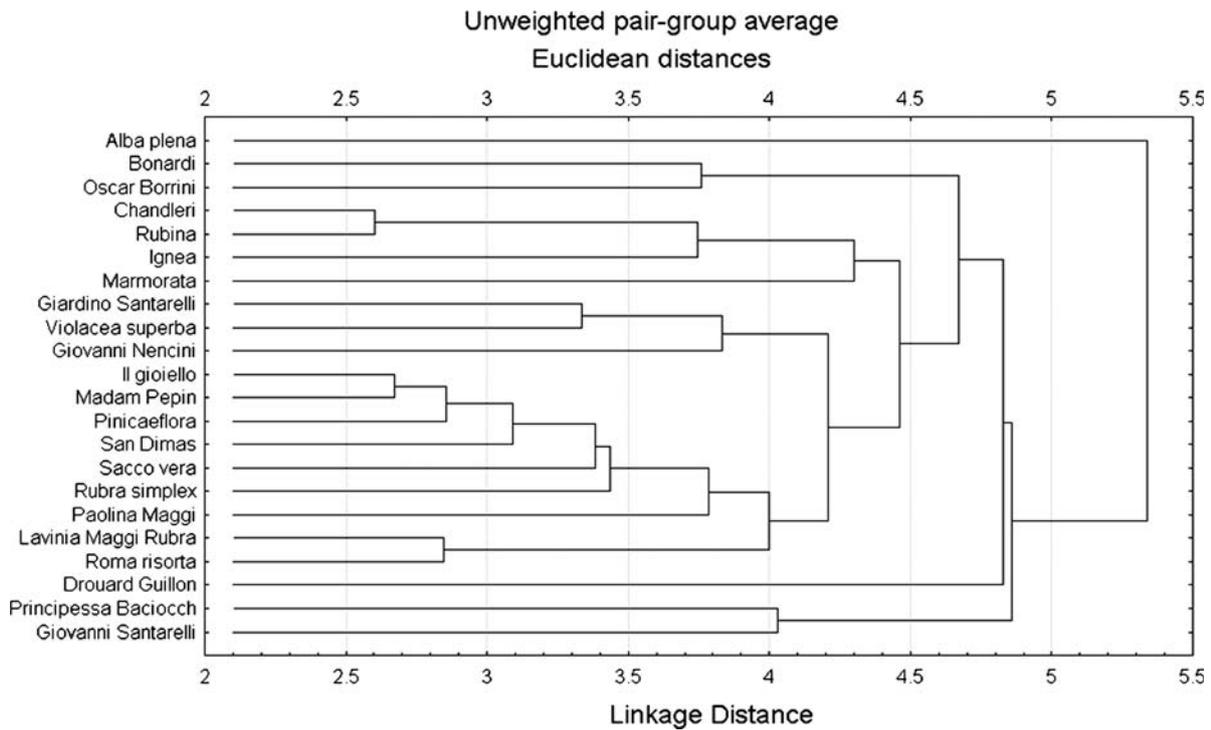**Fig. 8.** Prediction phase output for 'Chandleri'

Fig. 9. The UPGMA cluster analysis of *Camellia japonica* accessions

'Chandleri' has been selected in 1919 by Chandler in the nurseries of Vauxall, while 'Rubina' was selected in New York by Harrison and introduced in Europe in 1839. Another hypothesis about their high level of similarity lead to an inversion of their names during the past years. The old collection of ornamentals often suffers from homonymy and synonymy, resulting in an uncertain classification; to overcome this problem, the addition of more inputs to the network could be considered. For example, an useful parameter for the discrimination between 'Lavinia Maggi Rubra' and 'Roma Risorta' could be represented by the flower colour and/or form, as 'Lavinia Maggi Rubra' has red flowers, while in 'Roma Risorta' the flowers are pink-striped white. Though useful, this choice contains a negative aspect: the need to collect the plant material during the blooming, so reducing to one month the useful working time for *Camellia japonica* L. The kind of network needed should be assessed during the preliminary planning phase: a network exclusively based on leaf (phyllometric and fractal) parameters to be effectively used all over the year as *Camellia japonica* is an evergreen, or a more detailed and informative network with a greater number of parameters, but not available in any period of the year. The results obtained in this research show that backpropagation neural networks could be effectively used to discriminate *Camellia japonica* cultivars through the use of phyllometric and fractal parameters. Although further research is still necessary, these are of potential use in herbaria and museums, or even in the field, using simple dedicated instruments, such as a personal computer and an easily available optical scanner. Though one of the acknowledged advantages of the BPNN is the capability to overcome the need for a statistically representative sample of a population, for a real practicable system the changes in morphology caused by the environment should be taken into consideration and can't be excluded. As a matter of fact, further research should be addressed to assess the stability of the BPNN with camellia samples coming from very different environments.

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