Artificial Neural Networks Technology to Model and Predict Plant Biology Process

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1. Introduction

The recent and significant technological advances applied to biology places the researchers in front of an unprecedented new influx of large data set from different levels as genomics, transcriptomics, proteomics, metabolomics and ionomics (Hirai et al., 2004; Belostotsky & Rose, 2005; Schauer & Fernie, 2006; Kliebenstein, 2010). Thousands of data sets including millions of measurements have been generated, and moreover, most are freely available for plant researchers worldwide from plant specific databases, as for example the whole sequencing of different plant genomes like rice, *Arabidopsis*, poplar, papaya, grapevine and others... (Jaillon et al., 2007; Ming et al., 2008; Brady & Provart, 2009). There is a wide concern of integrating molecular, cellular, histological, biochemical, genetic and physiological information in plant biology (Katagiri, 2003; Thum et al., 2003; Trewavas 2006; Boone et al., 2007; Álvarez-Buylla et al., 2007) and also in other related fields such as crop improvement (Hammer et al., 2002), ecology (Hilbert & Ostendorf, 2001; Jimenez et al., 2008) and biological engineering (Huang, 2009).

Biological processes are both time variant and nonlinear in nature, and their complexity can be understood as the composition of many different and interacting elements governed by non-deterministic rules and influenced by external factors (Coruzzi et al., 2009, Gago et al., 2009). Commonly, most of biological interactions cannot be elucidated by a simple stepwise algorithm or a precise formula, particularly when the data set are complex, noisy, vague, uncompleted or formed by different kind of data (Prasad & Dutta Gupta, 2008; Gago et al., 2010a). It is important to point out that many times the behaviour of a biological system over a time period is difficult to understand and interpret and additionally, genetic and environmental factors show a very high degree of intra- and inter-individual variability, yielding a wide spectrum of biological responses (Karim et al., 1997; Guégan et al., 1998).

The Scientific community agrees with the idea that plant biology requires more efforts in developing platforms to integrate multidimensional data and to derive models for describing biological interactions in plants (Kitano, 2002; Hammer et al., 2004; Struik et al., 2005; Tardieu, 2003; Yuan et al., 2008; Brady & Provart, 2009). In this sense, more efforts are recommended to shift our view from a reductionist way to a systems-level view. This concept can be illustrated by the Coruzzi & co-workers (2009) example of the painting "La Grande Jatte" by the

pointillist artist George Seurat. If one stands near the image each of its strokes can be distinguish but cannot appreciate the beauty of the whole composition. Only from far away is possible to admire the beautiful sunset over the Seine in Paris. Most researchers are really on top of their topic, but far away from the whole view of the global subject. However, the future in plant research requires a broader view of biological plant systems, from the new available molecular and cellular discoveries to the whole-plant improvements (Kirschner, 2005; Yuan et al., 2008) or the performance at the crop level (Kitano, 2002; Wang et al., 2002), and even to the agroecological level (Jiménez et al., 2008; Huang, 2009).

Performing a meta-analysis of the data set of a whole system is not an easy task. Hammer & collaborators (Hammer et al., 2004) expressed the present research requirement, comparing it to the paths of navigation the early sailors needed to determine to be able to arrive at their destiny with accuracy. In the 18th century this scientific problem was solved with the development of appropriate tools to predict the longitude and latitude in the middle of the oceans. Thus, these authors proposed an interesting conclusion: researchers need the equivalent to sailor's tools to navigate across the different levels of biological organization from gene to phenotype. As Tardieu (Tardieu, 2003) has pointed out that as in all "marriages of convenience" the first contact is always difficult between the ones having the tools, the modellers, with an essential mathematical and physical background, and the others, in this case the plant biologists, having the data and the knowledge in plant physiology (from molecular to whole plant and from individual cells to whole populations) who necessarily have to work together to obtain this exciting new challenge.

Since the spectacular development of computers, researchers have been attempting to create non-biological entities that can imitate human level of performance. Such attempts have manifested in the emergence of a cognitive approach termed as artificial intelligence (AI) (Legg & Hutter, 2007). In 1956, John McCarthy defined this term as "*the science and engineering of making intelligent machines*", and currently textbooks define this field of computer science as "*the study and design of intelligent agents*". AI achieved higher popularity in the 90s and early 21st century was introduced as a new tool in different scientific and technical fields (Russell & Norvig, 2003). Since then, successful studies have been carried out using different techniques, such as artificial neural networks, fuzzy logic and genetic algorithms, which can combine and complement in multiple ways and have been used in many industrial and commercial applications (Taylor, 1996) such as: character speech or image recognition (Hussain & Kabuka, 1998; Ma & Klorasani, 2004), chemical research (Cartwright, 1993; Zupan & Gasteiger, 1993), process modelling and control (Lennox et al., 2001), and in pharmaceuticals (Rowe & Roberts, 1998; Shao et al., 2006) or biomedicine (Hudson & Cohen, 2000). Finally, also since the late 90s artificial networks have been used in some biological areas, such as ecology or environmental sciences (Lek & Guégan, 1999; Hilbert & Ostendorf, 2001; Huang, 2009).

Since the basis for understanding the theoretical and practical approaches, of these technologies, to the development of models and their applications to specific problems, has been review elsewhere (Müller et al., 1995; Rowe & Roberts, 1998; Hudson & Cohen, 2000; Huang, 2009) and in other chapters of the present book. Therefore, the purpose of this chapter is to review the topics relevant to AI technology, mainly genetic algorithms and fuzzy logic, and more extensively, neural networks for the integration of multidimensional data into models (networks) and the application of these models in addressing questions (decision making) in plant biology.

As plant biology researchers are not commonly used to these new technologies; we will begin with a brief introduction for the readers on their fundamentals in order to facilitate the understanding of its applicability.

2. Analysis of biological data

Data from plant biology are inherently complex. The types of data must be taken into account since they influence the kind of analysis to be carried out. Normally, plant biology data can be classified as binary data, those with only two possible responses, generally yes/no, i.e. survival (alive–dead); discrete data, which have more than two responses (which can take one of a finite set of values), i.e. number of proliferated shoots or flowers in an inflorescence: 0, 1, 2, 3…); continuous data, which have any response (which can take any of an infinite number of values, i.e. weight rates of the proliferated shoots or the flowers…). Less frequent data in the plant biological process are image data (β-glucuronidase (GUS) and green fluorescence protein (GFP) histological analysis in transformation experiments); temporal data (a particular sequence of events: phenological development of fruits; duration of time for the fruit growth; or fruit fresh weight gain or loss per month), time series data (chilling hours in different seasons and their effect on bud formation) or fuzzy data (some processes or physiological states in plant science are described by linguistic tags. Using fuzzy data: for example, the embryo developmental stages such as the different sets can be explained as globular, torpedo, heart; or the callus colour, can be classified as: brown, brownish, yellowish during a plant in vitro culture callogenesis process; see fuzzy logic section for a complete description). All these types of data can be included in a neural networks systems database, but the precision and accuracy of the number of data must be taken into account.

Over years, experimental designs and statistics have been important research tools for the plant researchers. Conventional analytical tools including logistic regression (for binomial and multinomial); Poisson regression (for discrete data), analysis of variance (ANOVA) for data only normally or approximately normally distributed continuous data to extract conclusions from data and to understand biological process have generally been used (Mize et al., 1999; Gago et al., 2010a). These techniques have allowed many questions to be solved, however many shortcomings can also been pointed out. Relevant difficulties are found when researchers need to consider a large data set with different kinds of data at the same time, to model the whole process studied or when the non-idealities of plant science processes do not conform (Hammer et al., 2004; Prassad & Dutta Gupta, 2008; Gago et al., 2010a). Such problems need a different sort of intelligence and connectionism. The kind of approach that shows through nodes and connection diagrams of the interaction and integration of multiple components in organisms is possible at present using computational models (Rumelhart & McClelland, 1986; Yuan et al., 2008; Huang, 2009).

3. Modelling plant biology process

Since the purpose of modelling is to increase our understanding of a plant science process (for example: providing understanding of the regulatory networks controlling developmental, physiological or other processes in plants) plant biology models to approach the complexity of these processes are needed.

To solve this requirement, an abstraction is needed which is able to organize the factors (inputs) and the parameters measured (outputs) into a functional model. These principles are really useful to approach the biological complexity, by abstracting and focusing on the relevant factors to obtain a broad view of the whole system and become essential for the understanding principal questions and decision support. To explain the concept of how the plant biologists can approach the biological complexity, an interesting example of what is needed of the abstraction is as follows: in 1736 an old mathematical problem "The seven

bridges of Königsberg", a city in Prussia (actually Kaliningrado in Russia), was negatively resolved by Leonhard Euler, and meant the beginning of the mathematical graph theory. This city was crossed by the Pregel river and had two islands in the middle, connected by seven bridges to each other and to the mainland. Would it be possible to find a way through the city by crossing each bridge once? Euler reorganised the problem in abstract terms: giving no importance to the city or if the bridge was made of wood or stone... only to reducing all the information to nodes/vertex (land masses) and edges/link (bridges). The answer: "It would be not possible"; however, Euler´s success was in reducing the problem to the significant inputs/factors and their relationships avoiding irrelevant data for the final purpose and to promote a suitable analysis.

Mathematical and computational models have been dramatically increasing in recent years in biological related sciences as in ecology (Stollenwerk et al., 2001; Anderson & Jensen, 2005), environmental and biodiversity conservation (Williams et al., 2004), epidemiology and pathogenesis (Brauer & Castillo-Chavez, 2001), genetic and biotechnology (Bar-Joseph et al., 2003), evolution (Nijhout et al., 2003) and animal (Schuster et al., 2005; Tracqui, 2006) or plant biology (Kovalenko & Riznichenko, 2007).

There is a wide variety of mathematical and computational models. Normally they are based in algorithms developed by theoretical and applied mathematicians, physics and/or bioinformatics, or for people with a firm background in theoretical biology, biological chemistry, mathematical biology in collaboration with informatics, computer science, physics, and/or engineering departments. In conclusion, mathematical modelling of biological systems generally requires a wide variety of methods and skills from multiple disciplines (de Vries et al., 2006).

For developing any mathematical model it is necessary to follow different steps: a) identification of the problem or process to be simulated, controlled and/or optimized; b) selection of data, variables (input and outputs), and very importantly, what is the model for; c) introduction of the accurate and precise data according from each variable, selection of equations (mainly algorithms) and the type of model: white box (all information is mostly available) or black box (no a priori information is available). If the black box model is chosen, some parameters can be used to fit the model to the system (in neural network the optimization of those parameters is called training); and d) model evaluation (normally cross-validation), to check the distances between the observed and predicted data (which should be as low as possible). The validity of the model is not only about if it fits well or not to empirical observations, but also about its ability to provide new insight which is partially occluded in the data, and which can not be known from direct observation or from statistical data analysis of the process. If the purpose is not achieved, the model is probably unnecessary, time consuming and useless. Another important issue to take into account is the quality of data. The model will be excellent if data are excellent. Models do not produce miracles. Bad quality data (non accurate, disperse or non precise) are not the best option to fit a model, especially if they are selected for a black box model, as neural networks are.

4. Artificial Neural Networks

Artificial Neural Networks (ANNs) are computational systems that simulate biological neural networks and they have been widely described in previous chapters of this book and in detail elsewhere (Russell & Norvig, 2003; Rowe & Roberts, 2005), but to better understand their application to plant biology it is pertinent to briefly review the way it is believed to function.

The main elements of biological neural networks are the neurons, electrochemical excitable cells that can receive signals or stimuli from other neurons via synapse connection (Fig. 1). The stimuli is received through the dendrites and transmitted to the cell body. If the stimulus is intense enough, the neuron generates another stimulus that is transmitted along the axon to the next neuron via synapses.
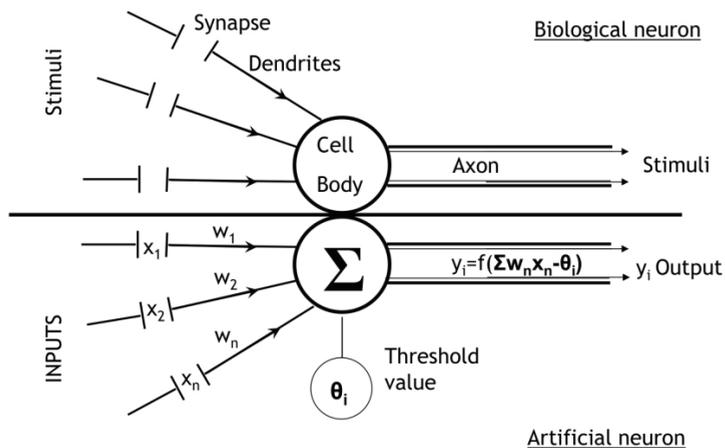
Fig. 1. Basic comparison between a biological neuron and an artificial neuron. X= input variable; W=weight of i_n input; θ= internal threshold value; f=transfer function.

The artificial neural network architecture is an interconnected assembly of individual processing elements called perceptron: "single nodes" or "artificial neurons" (Fig. 1). Each artificial neuron receives one or more inputs from neighbouring nodes, process the information and produces an output to be transmitted to the next node. The strengths of connections between two units are called "weights" which must be defined by the computational approach to solve or interpret a given problem (Takayama et al., 1999). While computing the output, the input information (X_i) is weighed either positively or negatively. The computational approach must also assign an internal threshold value (θ) to simulate the output action. At each node, the input values (X_n) are multiplied by their associate weight (W_n) to give a result, which is adjusted by its threshold value. The output is then determined using the non-linear weighted sum as the argument in a function "f" termed *transfer function or activation function* (Fig. 1; eq. 1).

$$y_i=f \left(\sum W_n \text{-} X_n \text{-} \theta_i\right) \tag{1}$$

Among the functions that can be applied: linear, hyperbolic tangent or radial basis form, etc is the sigmoid function (eq. 2) and is the most commonly used. Sigmoid function, $f(y_i)$, is conducted to the following layer as an output value. Alpha is a parameter relating to the shape of the sigmoid function. Non-linearity of the sigmoid function is strengthened with an increase in α.

$$f(y_i)=1/[1+\exp(\text{-}\alpha \, y_i)] \tag{2}$$

By connecting several artificial neurons (many hundreds of thousands) or simple nodes a complex artificial neural network can be obtained. Figure 2 illustrates the topology of one of the most popular and successful network architectures, a multilayer perceptron (MLP) networks, consisting of three simple layers; one input layer, one output layer and with just one hidden layer.
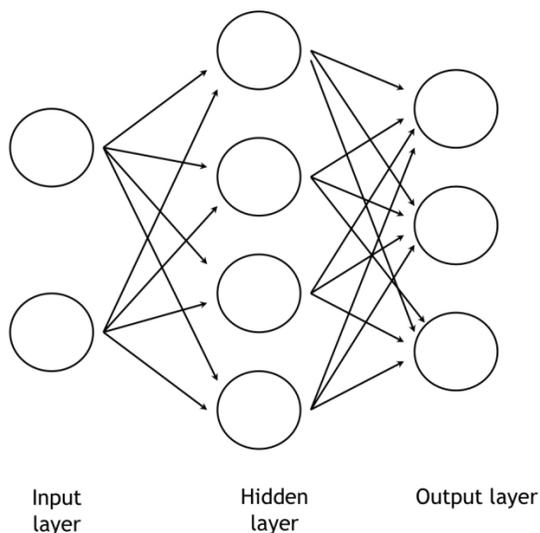

Fig. 2. A multilayer perceptron with one hidden layer.

ANNs is able to "learn" an approximate non-linear relationship between inputs and outputs using algorithms designed to alter the strength (weights) of the connections in the network to produce a desired signal flow. This "training" process is defined as a search process for the optimized set of weight values which can minimize the squared error between the data predicted by the model and the experimental data in the output layer (Takayama et al., 1999).

The ability of the network to memorize and process the information lies in the weights assigned to the inter-node connections, which determines the conductivity through the network. When the computed output is unacceptable, compared with experimental output, a back propagation process starts to modify several setting parameters (also called the learning rule) until the network attains a good generalization of the problem domain (Prasad & Dutta Gupta, 2008). The difference between actual and predicted outputs is usually quantified by means of an error function similar to those used in statistics.

In different research fields, it has been proposed that the performance of a well-designed MLP network is comparable to that achieved by classical statistical techniques (Rowe & Roberts, 1998) and therefore, suitable for a wide range of applications including: classification (Glezakos et al., 2010), pattern recognition (Frossyniotis et al., 2008), prediction on time series (Müller et al., 1995) interpolation (Gago et al., 2010a), and modelling complex systems with non-linear behaviour (Karim et al., 1997; Mehrota et al., 2008; Gago et al., 2010b, c)

It is important to point out that the strength of ANNs lays on its ability in detecting and quantifying complex non-linear relationships between inputs and outputs as well as its capability on generalizing distorted or partially occluded patterns (Taylor, 1996; Shao et al.,

2006). This powerful technology has also limitations, mainly related to the difficulties of interpreting the results in simple form or to elaborate formal reasoning or extract general rules (Colbourn, 2003). Complex "black box" models derived from ANNs technologies from a large data set with an important number of inputs could be difficult to analyze using general 2D plots or even 3D graphs. In order to avoid those limitations ANNs are usually combined with other AI techniques as genetic algorithms or fuzzy logic technology giving hybrid systems the results of which are easier to interpret and generalize (Plumb et al., 2005; Shao et al., 2006; Shao et al., 2007; Gago et al., 2010d).

4.1 Artificial Neural Networks as an alternative to traditional statistics

As pointed out by Mize and coworkers (1999), an extensive review of the literature shows that the use of correct statistical tools is not widespread in plant science. Many papers can be found where authors treat discrete or binomial variables as continuous variables by using the ANOVA. Moreover, when analyzed correctly, usually multiple comparison tests are performed to determine which of the factors studied have a significant effect on certain parameter or process.

The use of modeling and optimization techniques is even more restricted, generally being reduced to fit data to a specific functional form (linear or quadratic). This is because, in many cases, the plant researcher lacks the appropriate mathematical background for the analysis and his/her interaction with experts in statistics is not very fruitful. In this situation the use of a different technology as ANNs can be of great help.

Recent studies have demonstrated that AI technologies show the same or even better performance than traditional statistics for modelling complex non linear relationships hidden in the data and offer superior prediction powers (Landin et al., 2009 and references therein; Gago et al., 2010a).

From a formal point of view ANNs show several advantages over statistics: a) they can process different types of data together (continuous, binomial, discrete); b) they can be used to produce complex models without the previous knowledge of the functional form of dependence, so they can discover subtle relationships in the data; c) they do not require specific experimental design, being capable of using incomplete data, data acquired during a series of trial-and-error experiments or even historical data (Colbourn, 2003; Colbourn and Rowe, 2005).

Moreover, the use of ANNs does not require a specialized background and is a friendly technology, easy to use, that allows the modelling process with a limited number of experiments and costs, and makes inference of the combination of factors studied possible to obtain the best result (Gago et al., 2010a).

4.2 Applications of neural networks to plant biology

There are not many references in the literature on the applications of ANN to plant biology. This fact is more relevant, if we compare with other related areas as pharmaceutical science of important research in the last few years (Achanta et al., 1995; Colbourn, 2003; Takayama et al., 1999; Shao et al., 2006; Landín et al., 2009), ecology (Guégan et al., 1998; Hilbert et al., 2001; Adriaenssens et al., 2004) or agriculture (Huang, 2009 and references therein).

Pioneer studies in plant science deal with the use of AI technology to improve and/or optimize biotechnology processes production. An early work (Fukuda et al., 1991) explored the use of artificial neural networks to recognize live or dead plant cells by image processing. Other authors have also investigated the capabilities of image analysis of

somatic embryos developmental stage by neural networks (Uozumi et al., 1993) and image analysis of shoot length of regenerated rice callus using a hybrid (artificial neural networks/fuzzy logic) technology (Honda et al., 1997). Additionally, works dealing with modelling the bioproduction of Ginjo sake were carried out using a fuzzy neural network in order to control the processes in bioreactors (Hanai et al., 1997).

During the last twenty years there has been an increasing interest on this technology in the agricultural and biological engineering fields (Huang, 2009). Firstly, it was applied to model food quality (Whittaker et al., 1991; Eerikäinen et al., 1993) or fruit colour (Thai & Shewfelt, 1991) and, more recently, to manage herbicide application (Yang, 2003), yield estimation (Kaul et al., 2005; Khazaei et al., 2008), and water stress (Ondimu & Murase, 2008).

Neural networks were used for modelling crop yields on the basis of environmental conditions and pest control treatments in order to improve production. The optimization of pesticide concentration and periods of treatments to be used has a great impact on the costs and toxic residual levels of agriculture products (Jiménez et al., 2008). Health and economy are the important issues in the agricultural production nowadays. Different authors also established the relationship between the factors and crop yield for corn, sugar beet, soybean and winter wheat in order to help on decision-making processes (Kehagias et al., 1998; Kaul et al., 2005; Green et al., 2007; Jiménez et al., 2008).

Other authors have described the usefulness of ANNs for modelling the distribution of vegetation in past, present and future climates coupled with GIS (geographic information system). They have provided worthy contributions to understanding and conservation of these areas, especially when more detailed biogeographical data were available (Hilbert & Ostendorf, 2001).

More recently, detection of plant viruses has been carried out through a Bioelectric Recognition Assay (BERA) method in combination with neural networks (Frossyniotis et al., 2008). The sensors monitor the electric signal of the cells interacting with viruses making their identification possible. ANNs were trained with the responses of the biosensors to obtain a classification model of the culture cells infected.

ANNs based modelling approaches have also been applied in cell culture practice (Prasad & Dutta Gupta, 2008). One of the most important topics in *in vitro* culture is related with the supply of carbon source, commonly sucrose, to determine the effect on plant growth and physiological parameters. Tani and co-workers (1992) developed a growth model for *in vitro* shoots of alfalfa describing the effects of CO_2 inside the culture vessel and the sucrose content. This model increases the understanding of *in vitro* processes in a non-deterministic way. Three years later, a comparison between the deterministic mathematical model Extended Kalman Filter approach and ANNs was performed (Albiol et al., 1995). Authors stated the usefulness of neural networks for modelling at less cost, time and a smaller dataset.

Pattern recognition and classification models are commonly applied in plant tissue culture studies (Prasad & Dutta Gupta, 2008). Usually, the selection of embryos inside the embriogenic cultures is laborious, cost intensive and time-consuming. An image analysis pattern recognition system was developed by Zhang and coworkers (1999) using ANNs to select embryos of Douglas fir: the contour of embryo images was segmented, digitalized and converted into numerical values after the discrete and fast Fourier transformation (values obtained were higher than 80% for normal embryos). In another study, embryos of sugarcane from callogenic culture were selected by image analysis using machine vision analysis (MVA) confirming the technique as a rapid, non-invasive method for qualitative evaluation and quantification of *in vitro* regenerated plantlets (Honda et al., 1999). Finally, regenerated

plantlets could be sorted using their photometrical behaviour of their leaves in Red, Blue and Green colour regimes combining image analysis and ANNs (Mahendra et al., 2004).

Transference to field the micropropagated plantlets is a typical bottleneck in micropropagation protocols. Optimizing rhizogenesis and subsequent acclimatization is highly recommended to improve efficient propagation yields (Gago, 2009). The quality and the functionality of the root system, the vigour of the plantlets and other physiological status will be responsible for the quality of the plantlets in *ex vitro* conditions (Gago et al., 2009). Recently (Gago et al., 2010b), ANNs were used to model *in vitro* rhizogenesis and subsequent acclimatization data simultaneously of grapevine *Vitis vinifera* L. cv. Albariño. Studied inputs/factors (cultivar, IBA concentration and exposure time to IBA) showed significant effects on root number, *ex vitro* leaves, number of nodes and height of the acclimatized plantlets, the exposure time to the synthetic auxin IBA being the more relevant. The model allowed optimal predictions for every studied cultivar. The knowledge derived through ANNs can be easily increased by training the model by adding to the database new inputs (salt concentration, type of medium, other plant hormone, etc.) and/or outputs (plantlets weight, chlorophyll and carotenes content, stomata analysis, etc.).

5. Genetic algorithms

Once an ANN model has been obtained it is easy to predict what will be the output for a specific set of inputs, or in other word to formulate "what if" questions obtaining accurate responses (Fig. 3). This consultation mode will provide insight into the process studied. However sometimes, the main research objective is to determine the combination of input parameters that will provide the optimum result, that is, an optimization process which means to formulate "how to get" questions on the best/highest...output. In these cases a different AI technique, the genetic algorithms, can be applied.
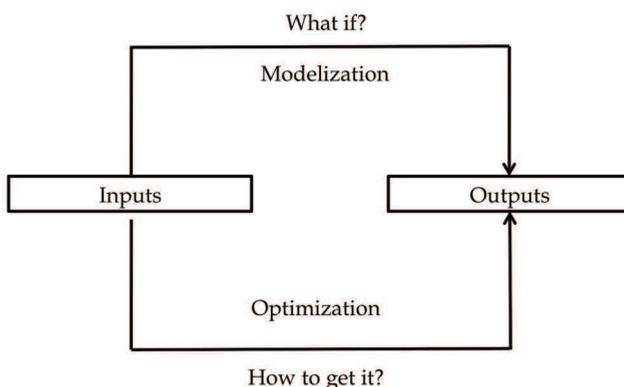

Fig. 3. The relationship between modeling and optimization from an ANN model (Modified from Rowe and Roberts, 2005).

"The genetic algorithm is an optimization technique based on evolutionary principles" (Cartwright, 1993). Genetic algorithms are based on the biological principles of genetic variation and natural selection, mimicking the basic ideas of evolution over generations. As Rowe & Roberts declare: "An optimization process evolves finding the best solution for a specific problem" (Rowe & Roberts, 1998). Genetic algorithm randomly generates a set of

candidate solutions to the problem. Solutions from one population are selected according to their fitness of evolving new populations to the problem. This is repeated until some condition is satisfied. After several generations an optimum must be achieved because the most suitable becomes the solution and therefore the more chances it has to reproduce. For a genetic algorithm to function it must possess several features. Firstly, a numerical description of how good a solution is to the problem. Secondly, a logical method of selecting individual solutions to become parents of the next generation must be fixed. And finally, a logical method of mixing the different elements to produce new solutions is necessary (Mitchell, 1998; Glezakos et al., 2010).

5.1. Applications of genetic algorithms to plant biology

Genetic algorithms have been used in plant science for different optimization processes. Noguchi & Terao (1997) have developed a mobile robot for harvesting fruit automatically and genetic algorithms were designed to find the optimal space solution for path planning.

Hybrid systems combining neural networks and genetic algorithms have also been used for optimizing the quality of fruits stored under a controlled environment (Morimoto et al., 1997; Morimoto & Hashimoto, 2000) and for plant virus identification through a Bio-Electric Recognition Assay (BERA) (Glezakos et al., 2010).

More complex *in vitro* culture processes such as shoot proliferation, root formation (rhizogenesis) and plantlets acclimatization have been modeled by ANNs and successfully optimized by genetic algorithms in woody fruit plants, such as kiwifruit (Gago et al., 2010a) and grapevine (Gago et al., 2010b).

6. Neurofuzzy logic

Human knowledge is typically built on linguistic tags (characterized by uncertainty or imprecision) and not on quantitative mathematical data. Many times words have higher significance in the real world than a collection of numerical data (Fig. 4) the basis being to solve problems, make decisions or draw conclusions.
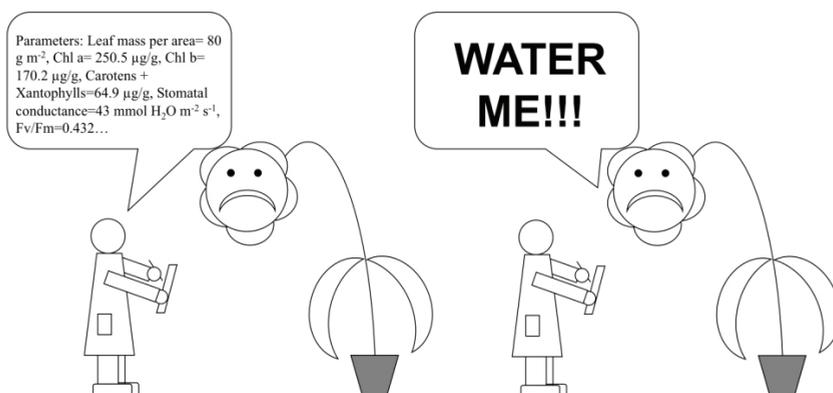

Fig. 4. Picture on the importance of precision and significance in the real world of plant research.

Neurofuzzy logic is a hybrid system technology developed to break in this topic. It combines the adaptive learning capabilities from ANNs with the generality of representation from fuzzy logic (Shao et al., 2006).

Fuzzy logic is an AI tool especially useful in problem solving. Fuzzy set theory was introduced by Zadeh (1965) as an extension of the classical set theory, which enables the processing of imprecise information using the membership concept (Adriaenssens et al., 2004). Prof. Zadeh illustrated the concept of fuzzy set and the degree of membership with the classical "tall man" example (Fig. 5). The conventional characteristic mapping of a classical logic set determines that a man is tall when his height is over 1.80 m. Zadeh extended the traditional definition of a logic premise from having just two extremes (either a man is over 1.80 m, so he is tall or lower 1.80 m, so he is not tall) to one in which there is a range in degree of truth from 0 to 1. For example new sets can be described qualitatively by terms as very low, low, average, tall or very tall. However, following the classical logic, a small difference of just 2 cm from 1.79 to 1.81 m, induces classification of the man in two completely different categories. This does not seem really "logic". But, using the fuzzy set theory, an element of those sets can be assigned to a fuzzy set with its membership degree ranging from zero to one, so two men 1.79 m and 1.81 m tall belong to fuzzy set "tall man" with membership degrees of 0.70 and 0.90 respectively (Fig. 5).
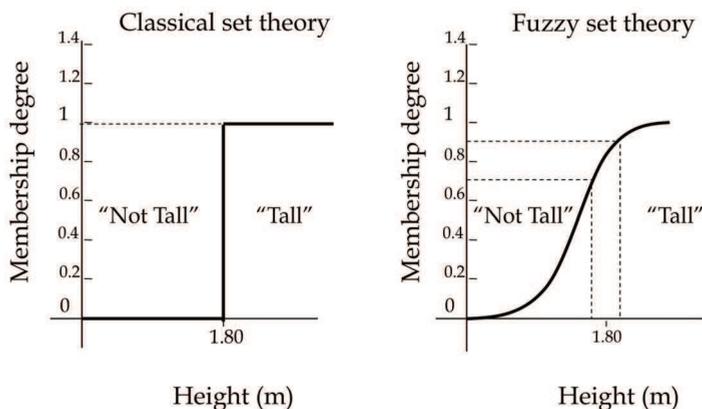

Fig. 5. Comparison between classical set theory and fuzzy set theory to illustrate the Zadeh´s example of the "tall man" (Modified from Zadeh, 1965).

These kinds of fuzzy data or fuzzy variables can be numerically characterized, but a fuzzification process is necessary. For better understanding of this concept, we will choose a typical tissue culture proliferation experiment in order to evaluate the effect of light intensity on the success of the proliferation of kiwifruit shoots. Light conditions for an *in vitro* culture experiment can be expressed by the Photosynthetic Photon Flux Density (PPFD) parameter (Fig. 6). The *x* axis is the PPFD with ranges for the fuzzy sets low, medium or high. The *y* axis represents the membership function and ranges 0 to 1 (also could be expressed from 0 to 100 per cent). It can be seen that a PPDF light of 80 mmol^{-2}s^{-1} can be regarded as both low and medium PPFD with membership functions of 0.7 and 0.4 respectively. In other words 80 mmol^{-2}s^{-1} is low light in a greater degree than it is medium light.

Therefore, fuzzy logic sets labels qualitatively using linguistic terms and also assigns varying degrees of membership called membership functions. The membership function
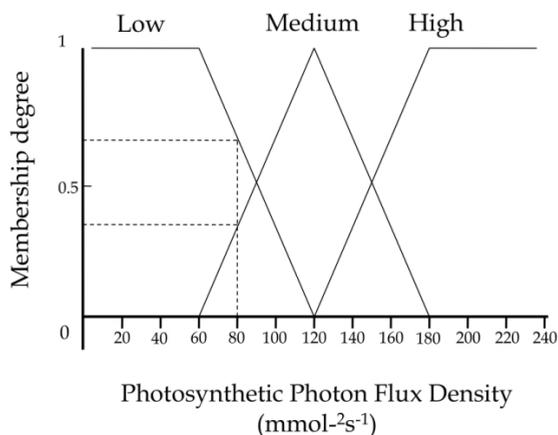
Fig. 6. Examples of fuzzy sets for light intensity (PPFD) for an *in vitro* plant tissue culture experiment.

then is subjective in nature and is a matter of definition rather than measurement. This process allows the interaction between linguistic terms (low, medium, high) and the membership functions making the terms meaningful to a computer. Additionally it makes expressing the behaviour of a system possible using natural language and enhancing the possibility of concise description of complex tasks or process.

In fuzzy-rule based systems, after modelling, knowledge is presented by IF-THEN rules. Fuzzy rules consist of two parts: an antecedent part stating conditions on the input variable(s), and a consequent part describing the corresponding values of the output variable(s). Given particularly values of the input variables, the degree of fulfilment of each rule is obtained by aggregating the membership degrees of these input values into the respective fuzzy sets. Going back to the example described previously if an evaluation of the effect of light conditions on the length of the plants in an in vitro culture experiment were carried out, the IF THEN rules could be similar to those presented in Table 1: IF PPFD is low THEN the plant length obtained is HIGH with a membership of 89% (more detailed information can be obtained in Gago et al., 2010d).

IF...THEN RULES		
IF PPFD is LOW	**THEN** Plant length is	HIGH (0.89)
IF PPFD is MID	**THEN** Plant length is	LOW (0.76)
IF PPFD is HIGH	**THEN** Plant length is	LOW (0.68)

Table 1. Examples of a fuzzy output using IF THEN rules describing the effect of the light intensity (PPFD) on the plant length in an *in vitro* culture experiment.

The fuzzy output is determined by the degrees of fulfilment and the consequent parts of the rules (Adriaenssens et al., 2004). The logical structure of rules facilitates the comprehension of a semi-qualitative manner, similar to that used by the human brain to analyze the real world (Babuska, 1998).

The major capabilities of fuzzy logic are the flexibility, the tolerance with uncertainty and vagueness and the possibility of modelling non linear functions, searching for consistent

patterns or systemic relationships between variables in a complex dataset, data mining and promoting deep understanding of the processes studied by generating comprehensible and reusable knowledge in an explicitly format (Setness et al., 1998; Shao et al., 2006; Yuan et al., 2008; Landin et al., 2009; Gago et al., 2010d).

Chen & Mynett (2003) have argued that definition of membership functions and induction of inference rules is the most difficult part in a fuzzy logic process. Fuzzy logic can be combined with neural networks to produce neuro-fuzzy techniques. Those hybrid systems combine the generality and flexibility of representation, a feature of fuzzy logic, with the powerful learning and adaptive capability of neural networks (Babuska, 1998; Adriaenssens et al., 2004).

6.1 Applications of neurofuzzy logic to plant biology

There are not many works in the literature on neurofuzzy logic in plant science. The neurofuzzy logic technology was used for controlling and modelling *Ginjo* sake brewing process: the interaction between sensory evaluation and the chemical composition of sake, beer and coffee was studied, showing this technology to have a high level of accuracy (Hanai et al., 1997). Also, there are some works related to the monitoring of herbicide sprayed in cornfields with a system that includes real-time image processing, weed identification, mapping of weed density, and sprayer control using a digital camera. Simulations using different fuzzy rules and membership functions indicated that the precision spraying has potential for reducing water pollution from herbicides needed for weed control in a corn field (Yang et al., 2003). Finally, hybrid systems as NUFZY involving a fuzzy approach and the training algorithm OLS (orthogonal least squares) has also been used to model accurately the lettuce growth and the greenhouse temperature (Tien & Van Straten, 1998).

Recently, Gago and coworkers (Gago et al., 2010d) compared the utility of the traditional statistical analysis and neurofuzzy logic technology for dataset highly complex and with great variability of direct rooting and subsequent acclimatization of grapevine. Neurofuzzy logic showed higher accuracy to identify the interaction effects between the factors: type of auxin (IBA, IAA and NAA), auxin concentration (1 to 50 mM) and sucrose concentration (0 to 9%) than conventional statistical analysis. Also, neurofuzzy showed a considerable potential for data mining and retrieve knowledge from the complex dataset. Understanding was increased thanks to IF-THEN rules generated from the model to facilitate researchers interpretation of the results, main effects and their consequences.

Considerable efforts have been made to understand artificial neural networks and neurofuzzy logic capacities in this sense, and many works are expected, in the near future, to provide a comprehensive insight into the expediency of processing networks in interpreting the database derived from plant biology research. In addition, one of the major advantages of these hybrid techniques is the capacity to model and estimate different complex processes.

7. Future perspectives

Biological systems are complex to understand. They have different scales of biological organization (genetic, biochemical, physiological...) and different factors influence them. Nowadays modern technology gives us the opportunity to generate a huge amount of biological data (Brady & Provart, 2009). This storm of information would be useless if at the same time the technology do not solve the problems associated of analysing, integrating and

extracting knowledge from those data. If the technology creates a problem, the technology should solve it.

AI technologies, the ones in use and the ones coming out in the future, will help the researchers with those difficult tasks: to integrate variable information, to explain what is going on, to model and finally to predict what will happen in a specific situation.

As described above neural networks can be used for a wide range of application domains in the general area of plant biology, and gives models as least as good as those obtained using statistical modelling. Neural networks combined with genetic algorithms can predict the combination of variables that would yield optimum solution when independent variables are fed into the network as it has been pointed out in this review. Neural network technologies have also spectacular advantages over other systems as the ability to capture non-linear relationships in the data (wherever their origin or type and even from incomplete data sets), without requiring prior knowledge from the user. In fact, the user does not need to have a deep mathematical or statistical background to employ effectively neural systems.

We think that once having overcome the natural reluctance of scientists to these new technologies, they will impose as the usual way for dealing with biological results. For that purpose, we strongly recommend the use of software packages, which incorporates visualization and data manipulation capabilities, within an easy to use interface, so the users do not need to be experts in neural computer (ie those used in Gago et al., 2010a, d).

Finally, the knowledge derived through neural networks can be easily increased by adding new data (inputs and/or outputs) to the database, giving new insight to understand the regulatory process controlling developmental and physiological processes in plants, as a whole. The knowledge obtained in this way should be crucial for both basic and applied plant biology.

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9. References

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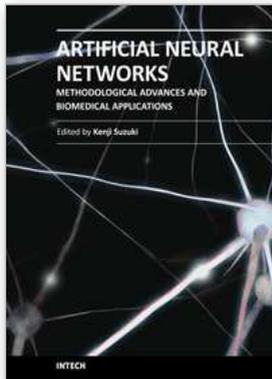
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Artificial neural networks may probably be the single most successful technology in the last two decades which has been widely used in a large variety of applications in various areas. The purpose of this book is to provide recent advances of artificial neural networks in biomedical applications. The book begins with fundamentals of artificial neural networks, which cover an introduction, design, and optimization. Advanced architectures for biomedical applications, which offer improved performance and desirable properties, follow. Parts continue with biological applications such as gene, plant biology, and stem cell, medical applications such as skin diseases, sclerosis, anesthesia, and physiotherapy, and clinical and other applications such as clinical outcome, telecare, and pre-med student failure prediction. Thus, this book will be a fundamental source of recent advances and applications of artificial neural networks in biomedical areas. The target audience includes professors and students in engineering and medical schools, researchers and engineers in biomedical industries, medical doctors, and healthcare professionals.

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