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Photometric Clustering of *In Vitro* Regenerated Plants of Gladiolus Using Fuzzy Adaptive Resonance Theory (Fuzzy ART) Neural Network

V.S.S. Prasad[1] • S. Dutta Gupta[1*]

[1] Department of Agricultural and Food Engineering, Indian Institute of Technology, Kharagpur 721 302, India

Corresponding author: * sdg@agfe.iitkgp.ernet.in

ABSTRACT

The application of Fuzzy ART as a clustering method is described to group regenerated gladiolus plants in terms of their similarity in leaf trichromatic features. The clustering results of Fuzzy ART were compared with the ART 2 modeling approach. The incorporation of fuzzy sets into the ART neural network enabled efficient clustering by refining grouping of leaf input patterns. The vigilance parameter considerably affected the number of generated clusters. A vigilance parameter of 0.91 was chosen as optimal into which the training and test set input patterns could be clustered into 7 and 5 distinct groups, respectively. The approach might provide scientists with a software sensor which can be used in selecting plants suitable for *ex vitro* transfer and in the quality control of micropropagation.

Keywords: artificial intelligence; gladiolus; image analysis; micropropagation; neural network; vigilance parameter
Abbreviations: ANN, artificial neural network; **ART**, adaptive resonance theory; **VP**, vigilance parameter

INTRODUCTION

In vitro environmental conditions have a significant impact on growth and quality of regenerated plants during micropropagation. There exists variation in environmental conditions in a culture vessel even under controlled conditions. Due to limited air movement in the culture vessel, there may be gradients in humidity and/or CO_2 concentration within the culture vessel. In addition, vertical light intensity and distribution also exists particularly in slender vessels (Ibaraki 2006). These might cause variations in the *in vitro* regenerated plants which ultimately affect uniformity in plantlet quality as well as percent survival upon *ex vitro* transfer. Such variations are not commensurate with that of well documented aspects of somaclonal variation (Larkin and Scowcroft 1981) and deserve attention for studies on *in vitro* behavioral aspects of the plantlets viz. rooting and storage organ forming ability, hyperhydric status and adaptability to *ex vitro* conditions. Since the physiological and behavioral variations among the regenerated plants are difficult to be resolved by human visual evaluation, machine vision coupled neural network based clustering might be an efficient alternative to select plants or group of plants suitable for *ex vitro* survival and also to assess the uniformity of the regenerated plants.

In plant tissue culture systems, artificial neural network (ANN) has been used for pattern recognition of somatic embryos, photometric assessment of regenerated plants and on-line estimation of biomass (Prasad and Dutta Gupta 2006). ANN-based modeling approach has been found to be more flexible, effective and versatile in dealing with non-linear relationships prevalent in cell culture practices. Recently, we have demonstrated the neural network aided image processing method for clustering of regenerated plants of gladiolus based on the trichromatic (RGB) features of leaves (Mahendra *et al*. 2004).

The present study is an extension of our neural network aided clustering approach by the incorporation of the fuzzy adaptive resonance theory model (Fuzzy ART). Fuzzy ART was introduced by Carpenter *et al.* (1991) for rapid stable learning of recognition categories in response to analog or binary input patterns. In terms of mathematical validations, Fuzzy ART yielded the most reasonable clustering compared to K means algorithm and self-organizing maps (Tomida *et al.* 2002). In Fuzzy ART, the resulting number of clustered groups depends on the similarity patterns of all inputs. The similarity pattern is characterized by the vigilance parameter ρ (VP). A learning procedure adjusted the weight vector W*j* of cluster *j*, where the vector represents the pattern of cluster *j*. In the present paper, we describe the application of Fuzzy ART to the analysis of photometric features of regenerated leaves for successful grouping of micropropagated plants of gladiolus and compare the clustering results to that of our previous work with ART 2 (Mahendra *et al.* 2004).

MATERIALS AND METHODS

The procedures for regeneration of plants and feature extraction from image histograms are described in detail in our previous clustering approach with ART (Mahendra *et al.* 2004). The leaf-derived meristemoid bud clusters of *Gladiolus hybridus* Hort. cv. Wedding Bouquet were cultured in a GA-9 vessel (Osmotek, Israel) with 50 ml of MS medium containing 0.5 mg/l NAA and incubated for three weeks to enable complete plantlet regeneration. The pH of the medium was adjusted to 5.6 before autoclaving at 121°C for 15 min. All the cultures were kept at 16 h photoperiod (irradiance of 60 μmol m^{-2} s^{-1}), temperature of 25°C, and relative humidity of 50%. There were five vessels number 1 to 5 each with five clusters. The training set comprised 25 leaf images each having its origin from a regenerated plant per cluster. Leaves collected from five clusters in a vessel were numbered 1 to 5 whereas the test data set was comprised of 13 leaf images representing arbitrarily selected regenerated plants.

Data thus obtained for both the training and test set were subjected to a neural network aided clustering analysis based on Fuzzy Adaptive Resonance Theory model (Fuzzy ART). **Fig. 1**

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illustrates the sequential steps of Fuzzy ART based clustering method.

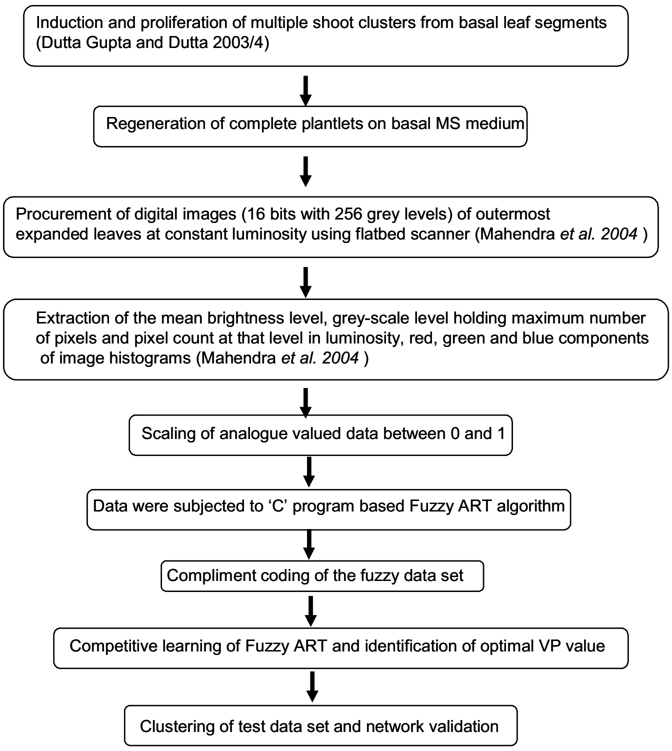

Fig. 1 Steps involved in Fuzzy ART clustering of regenerated plants of gladiolus.

Data normalization

The values of mean brightness level, maximum pixel count and grey-scale level for the maximum pixel count in the luminosity and trichromatic components of the leaf image pixels ranged from 0 to 255. Each input data point is scaled to a value lying between 0 and 1 as in Eq. (1):

$$\text{Normalized value} = 0.5 \times \frac{(\text{Original value} - \text{Mean value})}{(\text{Maximum value} - \text{Minimum value})} + 0.5 \quad \textbf{(1)}$$

Such data preprocessing is done to ensure the uniform statistical distribution of each input and output values and also to match the range of fuzzy neurons for efficient and fast functioning.

The structural architecture of Fuzzy ART is depicted in **Fig. 2**. The following description summarizes the working principle of Fuzzy ART. Each input pattern is presented as a 12 dimensional input vector representing 12 distinct properties of scanned leaf images (mean brightness level, maximum pixel count, grey scale level for the maximum pixel count in the luminosity, red, green and blue domains). These 12 values for each leaf pattern that are analogous in nature are complement coded. The complement of the datum x is 1-x. After complement coding, the leaf input pattern vector comprises of the original 12 data values along with their respective 12 complement coded values. Thus the input pattern vector becomes 2 x 12 dimensional. Data-scaling and complement coding converts the analogous valued scanned leaf image parameters into fuzzy data sets where the descriptive statistics and amplitude of the input information is retained. The fuzzy data sets are then subjected to Fuzzy ART clustering analysis. The dynamic computations in Fuzzy ART are determined by a choice parameter α (value is assigned >0; presently 0.01), a learning rate parameter (value is assigned >0 and <1; presently 0.1) and a vigilance parameter (value is assigned >0 and ≤1; presently 0.5 to 1.0).

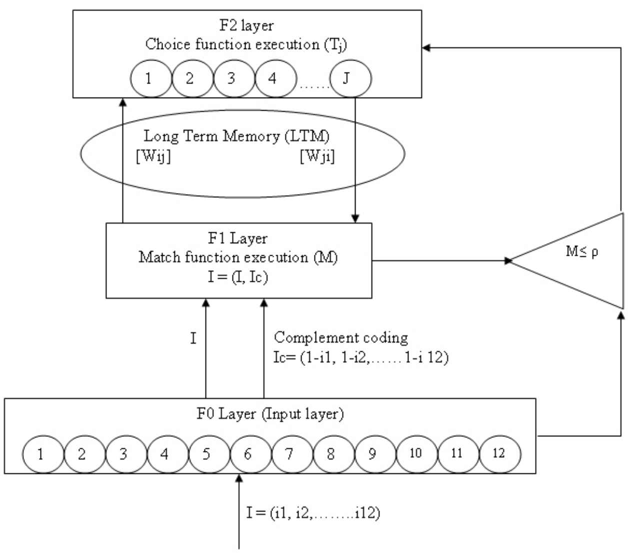

Fig. 2 Fuzzy ART module. The F0 layer contains 12 input nodes representing mean brightness level, Grey-scale level of maximum pixel count and maximum pixel count at luminosity and RGB regions.

Working principle of Fuzzy ART algorithm

When an input pattern is presented, a cluster category is chosen holding a maximum value for choice function (T*j*),

$$Tj\,(I) = \frac{|I \wedge wj|}{\alpha + |wj|} \quad \textbf{(2)}$$

The fuzzy and '$\wedge$' operator is defined as $(x \wedge y)_i \equiv \min(x_i, y_i)$, where,

$$|x| \equiv \sum x_i \quad \textbf{(3)}$$

An example is presented to illustrate the competitive learning of Fuzzy ART.

$I_{\text{leaf input pattern}}$ = | 0.72, 0.68, 0.89, 0.69, 0.61, 0.59, 0.67, 0.65, 0.70, 0.73, 0.75, 0.35 |

$|W_{\text{example}}|$ = | 0.27+ 0.71+ 0.89+ 0.30+ 0.38+ 0.41+ 0.32+ 0.65+ 0.29+ 0.26+ 0.24+ 0.64 |
= 5.36

| $I_{\text{leaf input pattern}} \wedge W_{\text{example}}$ | =
| 0.72 $\wedge$ 0.27 + 0.68 $\wedge$ 0.71 + 0.89 $\wedge$ 0.89 + 0.69 $\wedge$ 0.30 + 0.61 $\wedge$ 0.38 + 0.59 $\wedge$ 0.41 + 0.67 $\wedge$ 0.32 + 0.65 $\wedge$ 0.65 + 0.70 $\wedge$ 0.29 + 0.73 $\wedge$ 0.26 + 0.75 $\wedge$ 0.24 + 0.35 $\wedge$ 0.64 |
= | 0.27 + 0.68 + 0.89 + 0.30 + 0.38 + 0.41 + 0.32 + 0.65 + 0.29 + 0.26 + 0.24 + 0.35 |
= 4.83

T_{example} = 4.83 / (0.01 + 5.36)
= 0.89

The maximal T*j* is defined as the 'winner' cluster for the input pattern. When more than one T*j* is maximal, the output nodes become committed to cluster categories in the order of j = 1, 2, 3….
The match function of a cluster category is represented by Eq. (4):

$$Mj = \frac{|I \wedge wj|}{|I|} \quad \textbf{(4)}$$

Upon feeding a new input pattern, if the match function of a winner cluster category is less than the vigilance parameter value, then the mismatch reset occurs. Due to mismatch, a new cluster is generated that has the next maximal T*j* value according to the input vector.

Initially, all the weight vectors are assigned a value of 1.0. Therefore, the weight vector of a new cluster is as follows,

W_j (new cluster) = β [$I \wedge W_j$(initial)] = I

But in case the match function value exceeds the VP value, then resonance occurs and the current pattern is included in the winner cluster. Upon such resonance, the weights of the winner cluster are modified according to Eq. (5):

$$W_j\,(\text{new}) = \beta[I \wedge W_j(\text{old})] + (1-\beta).\ W_j\,(\text{old}) \qquad \textbf{(5)}$$

As large numbers of analog input patterns disturb the norm of weight vectors, complement coding is carried out to normalize the input vectors, while maintaining the amplitude of inherent information.

Complement of input pattern (Ic) = 1- Original input pattern

Upon complement coding input is fed to the recognition system as 2 × 12 dimensional input vectors. Clustering analysis was performed by Fuzzy ART algorithm compiled in 'C' language (Ver. 1.0) by Tomida *et al.* (2002).

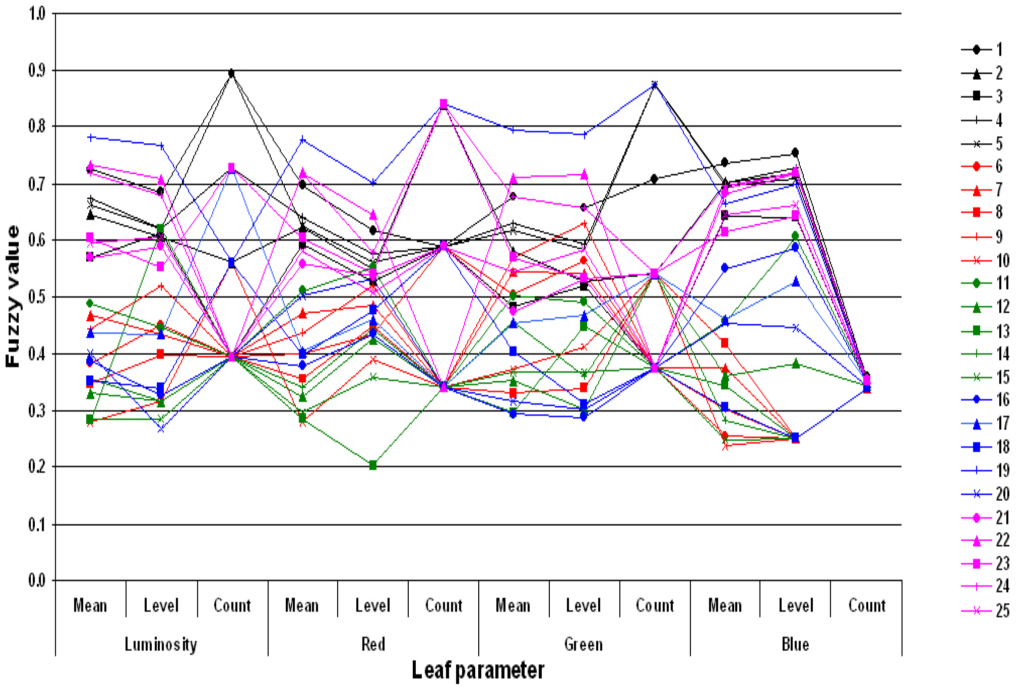

Fig. 3 Fuzzified data of training set representing luminosity and trichromatic features from 25 digitized leaf images. Leaf input patterns are numbered from 1 to 25.

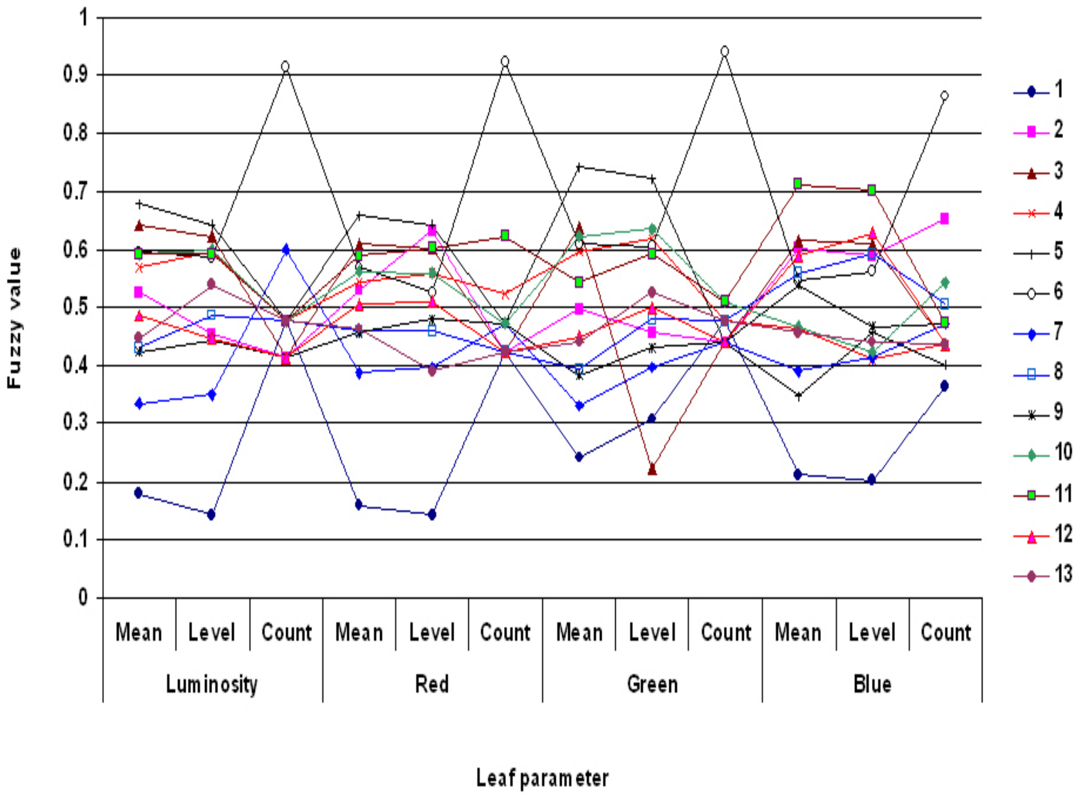

Fig. 4 Fuzzified data of test set representing luminosity and trichromatic features from 13 digitized leaf images. Leaf input patterns are numbered from 1 to 13.

RESULTS AND DISCUSSION

We used the previous data of luminosity and trichromatic features at RGB regions from 25 and 13 digitized leaf images as training and test set respectively for normalization. Normalized data thus obtained were used as the input data for the fuzzy operator. Fuzzified data of training and test set are presented in **Fig. 3** and **Fig. 4**, respectively. Data normalization is necessary to bring stability in the network performance and to prevent aberrant proliferation of the number of clusters. Initially, training set data of leaf images (**Fig. 3**) were subjected to Fuzzy ART clustering analysis with a vigilance parameter value ranging from 0.5 to 1.0 at an interval of 0.1 increments.

As per the theoretical assumptions of working principle of Fuzzy ART algorithm, the number of generated clusters will increase with higher VP values. It is evident from **Fig. 5** that at VP of 0.5 and 0.6 the number of clusters obtained was 1 but the number increased to 2 at VP of 0.7 and 0.8. Thereafter a steep rise in the number of clusters with a concomitant increase in VP was observed.

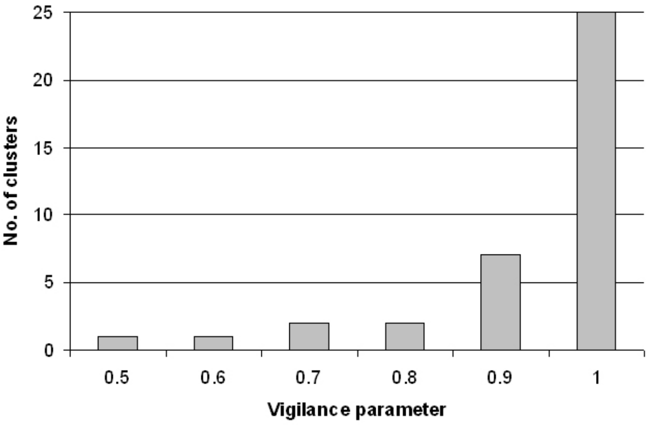

Fig. 5 Effect of vigilance parameter on the number of clusters generated by Fuzzy ART.

Further, the analysis was performed with increasing values of vigilance parameter from 0.8 to 1.0 at an interval of 0.01, in order to study the effect of vigilance parameter on clustering. As and when a higher VP is used, the value of match function tends to be comparatively lower which allows the mismatch reset to result in the generation of large numbers of new clusters. Up to a VP of 0.91, the increment in the clustering pattern was in the order of 1 to 2 clusters per unit increase in the VP value. With the VP value over 0.91 the clustering pattern showed an exponential trend with an increase of 2 to 3 clusters per unit increment in VP value (**Fig. 6**). The network displayed a higher sensitivity with the VP above 0.91. A similar kind of variation in clustering pattern with a change in VP value was also noted in the analysis of gene expression profile using Fuzzy ART (Tomida *et al*. 2002). In order to have reasonable, consistent and stable clustering, in the present study the VP of 0.90 and 0.91 were chosen due to the following reasons:

a) Stable clustering pattern up to VP = 0.90

b) Inconsistency in network performance with VP > 0.91 resulting in drastic variation in clustering pattern.

Both the VP values of 0.90 and 0.91 grouped the training data sets into 7 cluster groups (A-G). The plants of vessel number 4 were distributed into four cluster groups (C, D, E and F) with VP of 0.91, whereas VP of 0.90 clustered them into 3 groups as C, E and F (**Table 1**).

To check the efficiency of the learning process, the test data set comprising of image parameters of 13 leaves were subjected to Fuzzy ART aided clustering with the VP value of 0.91. The test leaf patterns were allocated into 5 groups. It is worthy to note that most of the training set patterns except a couple were distributed into the first five groups which suggests the similarity in the grouping pattern among the training and test data sets. It also indicates the efficiency of network classification and its ability to recognize the minute variation in leaf image properties. As compared to our previous work with ART2, the Fuzzy ART generated more clusters in both the training (7) and test (5) data sets. The incorporation of the fuzzy set theory into Adaptive resonance theory has resulted in the refinement of grouping pattern and thereby efficiently projected the variation among the regenerated plants in terms of leaf trichromatic features. The success in achieving high quality and uniform *in vitro* transplant production with high rate of organogenic ability to develop storage organs and *ex vitro* survival is largely dependent upon the ability to discriminate the variants. These microenvironment enforced variations are to a great extent inherent, dynamic and complex so much so

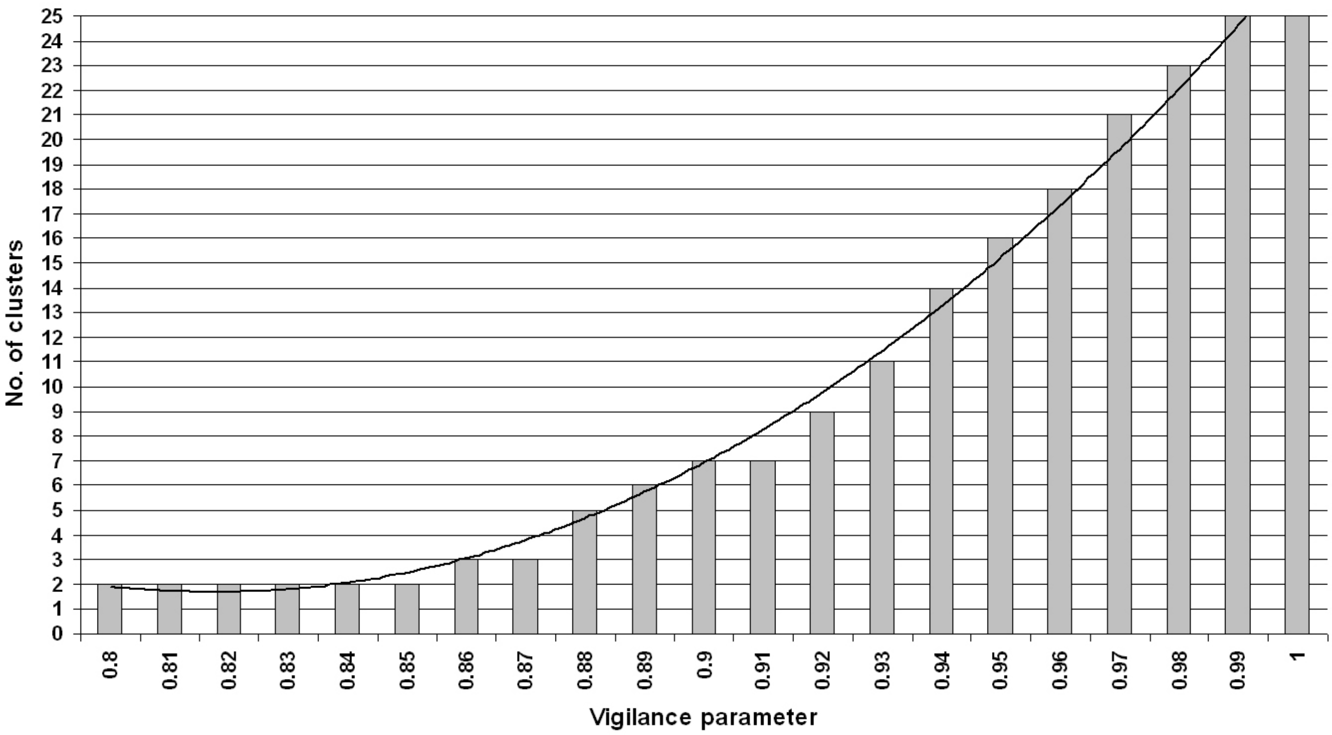

Fig. 6 Change in the clustering robustness of Fuzzy ART.

Table 1 Distribution and grouping of leaf input patterns by Fuzzy ART.

				VP = 0.90			
Training set				**Cluster group**			
Vessel No.	**A**	**B**	**C**	**D**	**E**	**F**	**G**
1	1,4, 5	2			3		
2			6, 7, 9	8, 10			
3				12,13,14,15	11		
4			18		16, 17, 20	19	
5		21,23,24,25					22
				VP = 0.91			
				Cluster group			
Vessel No.	**A**	**B**	**C**	**D**	**E**	**F**	**G**
1	1,4, 5	2			3		
2			6, 7, 9	8,10			
3				12,13,14,15	11		
4			18	16	17, 20	19	
5		21,23,24,25					22
				VP = 0.91			
				Cluster group			
	A	**B**	**C**	**D**	**E**		
Test set	1	2, 3, 8, 12	4,5,10,11,13	6	7, 9		

that conventional mathematical descriptors would not be sufficient enough to interpret them. A non-invasive Fuzzy ART neural network based approach appeared to be promising for the assessment of such typical biological variability. Neuro-fuzzy clustering algorithms were used extensively in characterizing the genetic and metabolic functions of prokaryotic organisms. Fuzzy ART has been used to successfully cluster sporulation specific gene expression profiles of *Saccharomyces* (Tomida *et al.* 2002). The clustering results were found to be mathematically and biologically consistent even with noised data. Fuzzy ART was applied to time series micro-array data of oxidative stress in *Saccharomyces* to infer genetic interactions (Takahashi *et al.* 2002, 2003). Kato *et al.* (2002) analyzed the gene expression of heat shock res-ponse using Fuzzy ART. Similarly a time course gene expression profile of *E. coli* was subjected to Fuzzy ART to infer the role of genes in organic solvent tolerance (Shimizu *et al.* 2005). In plant tissue culture systems, the application of a Fuzzy neural network was restricted only to the estimation of shoot length of regenerated rice (Honda *et al.* 1997).

The present work is an improvement over our previous study (Mahendra *et al.* 2004). Increase in the number of groups by Fuzzy ART over ART 2 algorithm suggests refinement in the clustering pattern. The extent of difference in photometric pattern was projected more efficiently with Fuzzy ART. The photometric behavior and their differences among the regenerated plants may be utilized in imaging photosynthesis of the micropropagated plants.

CONCLUDING REMARKS

In conclusion, we successfully applied Fuzzy ART for the first time as an efficient clustering method to group the regenerated plants of gladiolus based on their leaf trichromatic features. Such an approach might provide us with a software sensor to discriminate photometric variants among the regenerated plants which would be of use in selecting plants or group of plants suitable for *ex vitro* transfer and quality control of micropropagation.

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