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Predicting Cut Rose Stages of Development and Leaf Color Variations by Means of Image Analysis Technique

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Abstract

The monitor and prediction of crop developmental stages, particularly harvest time, play an important role in planning greenhouse cropping programs and timetables by cut rose producers. There have been many scientific reports on the application of image analysis technology in estimating greenhouse crop growth stages. In the present research, we studied leaf color variations over time by taking timely images from four commercial rose cultivars and processing them later using image j software in RGB color space. Results revealed a higher correlation between the leaf color variations and the stages of stem growth in both white color (R2=0.89) and colorful cultivars (R2=0.94). Furthermore, it was determined that there was a significant difference in leaf color components within stem layers in all cultivars. A good correlation was also observed between the leaf total chlorophyll measured directly by spectrophotometric method and the data acquired indirectly from SPAD readings. Among the models fitted to the stem height and color variation data, linear and exponential models performed best. However, some differences were observed between the cultivars. The potential observed in image analysis technique in detecting color differences among the leaf layers and its versatility in non-destructive determination of a link between the leaf color changes and rose stem growth give it the utmost merit and applicability in greenhouses. Developing such a model for other important cultivars of greenhouse roses will make it possible to equip rose greenhouses with several powerful and reliable tools in order to assist the growers to precisely adjust the crop harvest time and accurately plan their operations according to the market demand and policy.

Keywords: Chlorophyll, Cut roses, Image analysis, Leaf color, RGB model.

INTRODUCTION

Roses are one of the world's most important crops in the floriculture industry either as a garden plant or a cut flower (Buck-Sorlin *et al*., 2011). Image processing has been proved to be an effective tool for plant growth and development analysis in various fields of agriculture. The parameters like canopy shape, leaf color, leaf area, quality of product, stem length, and flower and leaf shape are among the most important characteristics usually used by researchers involved in ornamental horticulture. Some of these analyses may be expensive and may require some high-tech equipment beside the well-trained technicians to handle the experiments, especially those with potentially hazardous chemicals. Using digital image analysis to determine plant characteristics (color, shape, area, disease, etc.) could be one of the best solutions for growers to increase efficiency of greenhouse and reduce production labor cost (Berger *et al*., 2012). Digital image analysis has several advantages among which the speed of giving accurate response is the most important one (Stutte, 1990). Another significant advantage is that it makes it possible to image the same plant several times during the plant growth cycles without taking destructive samples which is common in many laboratory methods (Berger *et al*., 2012).

Monitoring the growth and development of crops in commercial glasshouses requires accurate quantification of a wide range of plant characteristics such as plant height, flower color, and leaf area infected by pests and diseases. Modern digital imaging technology is widely used for routine monitoring in industry (Brosnan and Sun, 2004). Recently, utilization of digital imagery has become a new trend in plant color analysis. Digital cameras in combination with computers and appropriate software can be used to photograph, scan, and evaluate leaves for color with relative ease and at an affordable cost. In agriculture, digital technology is used to acquire the information related to the physiological states of plants, including leaf area index (Liu and Pattey, 2010), chlorophyll content (Yadav *et al*., 2010; Dutta Gupta *et al.*, 2013), light intensity distribution on canopy surface (Ibaraki *et al*., 2012), root growth and development (French *et al.*, 2009; Lobet *et al.,* 2011), disease severity (Corkidi *et al.,* 2006; Wijekoon *et al*., 2008; Cui *et al*., 2010), and leaf shape variations (Iwata *et al.*, 2002; Keyser *et al.,* 2013). In rose plants this method has been used to automate rose cutting production in greenhouses (Noordam *et al.*, 2005), early pest detection in greenhouse roses (Martin *et al.*, 2009), detection of powdery mildew disease (Lopez *et al.*, 2011), estimation of flower number in rose (Adamsen *et al.*, 2000), roundness of rose flower shapes (Zhenjiang, 2000), and leaf area index (Shimomura *et al.,* 2003).

The color patterns of plant organs such as leaves, flowers, and fruits are one of the most important targets for plant improvement programs, as the color patterns of such organs are related to the quantity and quality of agricultural products (Yoshioka *et al*., 2004). Leaf color which is normally visualized by naked eyes has been used for evaluating the plant status and as a visual indicator of plant health (Townsend and McIntosh, 1993; Murakami *et al*., 2005) and nutrient status (Yuzhu *et al.,* 2011; Wiwart *et al.*, 2009) and gives a good indication of chlorophyll and other pigments content of leaves (Gaddanakeri *et al.,* 2007). Color is one of the main characteristics used in image analysis of plants.

Computer aided morphometric methods in quantitative shape analysis facilitate measuring and visualizing the differences in forms in a highly effective, reproducible, accurate, and statistically powerful way. Quantifying the plant phenotypic traits is an important step to group and classify plant varieties. Modern imaging techniques have high resolution and allow for the visualization of multi-dimensional and multi-parameter data. Imaging techniques are used to quantify complex traits related to growth and yield and to develop applications suitable to stress measurement and plant phenotyping in greenhouses (Berger *et al.,* 2010). In greenhouse cultivation, scheduling the production operations and activities is very vital when it comes to adjusting the harvest time with the market demand. Greenhouse roses are usually harvested either by flush or continuous method (Kool, 1997). However, continuous method with some harvest peaks in times of high demand by market is preferred and selected by most growers. In order to cope with the market fluctuations,

growers should not only follow some proper cultivation practices but they must also benefit from some tools to predict the amount and exact date of stem harvest. Despite of getting assistance of some mathematical models in predicting the harvest time (Pasian and Leith, 1996), they are not accurate enough and applicable in large scales. Image analysis techniques may provide an opportunity to monitor and digitize the whole greenhouse crop covered area and makes it possible to determine the crop developmental stage by analyzing the timely captured images. It was determined that new emerging leaves of some varieties of roses were in red color and then they would lose their redness or it would pale as they mature (Schmitzer *et al.*, 2009). This characteristic may offer a way to find a relationship between the developmental stages of the growing shoot and the degree of shoot foliage color change over time. In the present study, firstly we tried to measure the changes in the leaf pigmentations over time by taking timely images and finding a relationship between this trait and the stages of shoot development, and secondly evaluate the image analysis potential in detecting some visual and morphological differences among the rose plant leaf layers.

MATERIALS AND METHODS

Plant materials

The experiment was conducted in a research greenhouse located at the University of Tabriz (Tabriz, Iran). Four greenhouse cut rose cultivars namely, 'Caribia', 'Full House', 'Cherry Brandy', and 'Polar Star' were bought from a commercial greenhouse and then, they were transferred to the research greenhouse. Plants were selected at the same developmental stages and were planted in 6 L pots filled with a medium composed of cocopeat 70% and perlite 30%. Greenhouse condition was set in a way that it favored optimum plants growth under the natural photoperiod and temperature ranged from 18.0 ± 2°C to 28.0 ± 2°C with RH varied between 50% and 70%. Plants were fertigated with a standard nutrient solution prevailed in the region. Plants were trained according to the arching system by bending weak and unmarketable stems down. The experiment started on May 26, 2014 by bending down of two uniform stems above the first 5¬-¬leaflet leaf selected from each experimental pot and continued till August 26, 2014. The effects of two factors including cultivar at four levels and leaf layers composed of three levels (top, middle, and bottom section of the stem) were studied in a split plot design replicated four times with the cultivar assigned to main plot and leaf layer to subplot. The experiment data were obtained and recorded on a weekly basis and were subjected to statistical analysis at the end of experimental period.

Digital image acquisition

A SLR digital camera (Canon's EOS 550D, Japan) was used for taking photos. The camera was mounted and fixed on a height adjustable tripod. Photography was carried out under sunny solar noon days by putting a specially designed white matte plate under the subject (stem or leaf) with a 1 cm2 square attached to it as scale. The images were taken under the following camera settings: white balance: auto, shutter speed: 1/125 s, ISO: 200, focal length: 18 mm, and image resolution of 5184 in 3456 pixels. Three types of images were taken according to the type of the subject and the angle of view; stem plan view: the camera was put over the stem so that it could capture a full frame of stem; stem side view: the image was taken from side with a full shot of the entire stem; single leaf view: one leaf randomly selected from each leaf layer of the stem and after being laid out between two plates of clear glass, photos were shot. The images were saved in JPEG format with 256 grey levels per each color channel. The Image j software (National Institutes of Health, Bethesda, Maryland, USA; version 1.4, free download form http://imagej.en.softonic.com/) with the latest relevant plugins were used to analyze the images.

Image analysis

The taken images were then introduced to Image j software in order to undergo some prepa-

ration processes. Image pre-processing, segmentation, and background deletion were done by the methods previously explained and defined (Easlon and Bloom, 2014).

Leaf area

At the first step, foliage area was selected and separated from image background by segmentation method (Li *et al.*, 2010), and then we calculated the area occupied by plant leaves by dividing the total counts of pixels composing the leaf area by the total counts of pixels enclosed in the scale (1 cm^2) (Easlon and Bloom, 2014).

Color separation

After preliminary image processing, pixel values of each channel (Red, Green, and Blue) were extracted from the images and then they were normalized or average red (R), green (G) and blue (B) values of the RGB color model were calculated. Normalization reduces the effect of illumination (Cheng *et al.,* 2001). The estimated indices are listed and defined in Table 2. These average values were calculated by taking account of all the pixels within the leaf section. To get an estimation of greenness and normalize the variations observed in irradiance among the photos, green ratio (rG) was also calculated and averaged by photo (Eq. (1)):

$$rG = G / (R + G + B) \quad (1)$$

where, G = green, R = red, B= blue. A ratio was also calculated for the red (rR) and blue (rB) channels by Eq. 2 and 3 (Lee and Lee, 2013; Wang *et al.*, 2013).

$$rR = R / (R + G + B) \quad (2)$$

$$rB = B / (R + G + B) \quad (3)$$

Percent greenness was calculated as % green = 100 × [leaves green area/total leaves area], and percent redness was calculated as % red= 100 × [leaves red area/total leaves area]. Value of each color reports percentage of the color on plant surface. A typical image from the digital camera is shown in Fig. 1 (a). Fig. 1 (b, c and d) shows color channels and Fig. 1 (e) shows the image after the pixels were segmented into two groups. The calculated canopy cover was approximately the same in Figs. 1 (f) and 1 (g).

Stem height

Stem heights from each experimental unit were measured from side view images by taking the scale as an index to calculate the entire stem length.

Table 1. Color indices based on the RGB and normalized RGB values.

Color index	Definition
Red	Non-normalized red
Green	Non-normalized green
Blue	Non-normalized blue
Normalized red	rR = R / (R + G + B)
Normalized green	rG = G / (R + G + B)
Normalized blue	rB = B / (R + G + B)

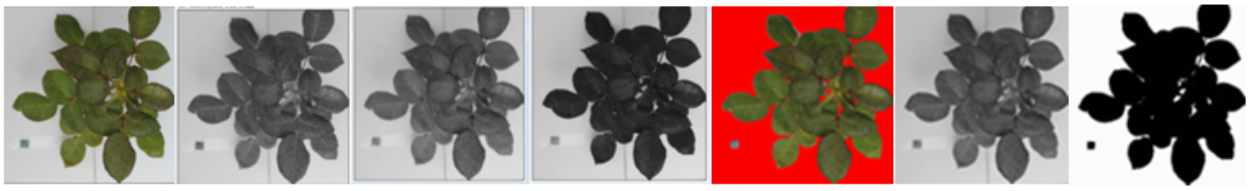

Fig. 1. a) Original color image, b) Red channel, c) Green channel, d) Blue channel, e) Image after removing background, f) Gray scale image, g) Binary image.

Chlorophyll measurements

Stems were divided into top, middle, and bottom layers. Then, two leaves were randomly selected from each section. Following this, the leaves were undergone three types of chlorophyll measurements: direct (spectrophotometric measurement) and indirect method (SPAD measurements and image analysis method) as follow:

Image analysis

At first, we took leaves photos from each layer to carry out image analysis. The RGB color space was used for chlorophyll estimation according to the procedure previously applied by some authors (Yadav *et al*., 2010; Ali *et al*., 2012). Six indices based on the RGB values were calculated for each leaf sample (Table 2).

Table 2. Summarized ANOVA for the measured traits within different layers.

S.o.V	df	Spect	SPAD	Cart.	R	G	B	R-B	R+G	R+B	R+B+G
Top layer											
Cul	3	769.5**	159.6**	637.3**	223.2**	384.9**	290.6*	10.07^{ns}	1183**	1017.6**	2643**
Error	8	10.015	0.72	5.33	27.38	16.9	41.58	3.9	86.1	133.9	242
Middle layer											
Cul	3	2326.3**	64.2**	422.7**	159.2**	207.25**	201.71*	12.86^{ns}	724.16**	709.14*	1678**
Error	8	19.48	0.311	14.38	9.43	13.62	24.49	9.61	34.52	57.35	105.01
Bottom layer											
Cul	3	993.3**	48.8**	66.06**	160.8*	122.5*	168.4*	2.35^{ns}	556.8*	656.1*	1322.4*
Error	8	16.49	0.432	4.87	31.28	24.6	37.4	4.15	106.3	133.4	266.2

*,** and ^{ns} indicate significance at P < 0.05, P < 0.01 levels and non- significance, respectively.

SPAD readings

Indirect chlorophyll measurement by chlorophyll meter for individual leaves with five successive readings was done by a portable chlorophyll meter SPAD-502 (Minolta, Japan). The measurements were averaged using the internal function of the chlorophyll meter. This device provides a quick and non-destructive method to estimate leaf chlorophyll content in plants.

Chlorophyll extracted with spectrophotometer

The total chlorophyll (TCHL) and carotenoids content were measured based on the method used by Arnon (Arnon, 1949) in the same leaves subjected to image analysis and SPAD method.

Experimental design and statistic

The experimental design was a randomized completely design with four cut rose cultivars as experimental treatments replicated four times and summed to 16 plots. The effect of stem layers on the color components of leaves was defined as a separate experiment. All the recorded data were analyzed by analysis of variance (ANOVA) procedures using Statistical Software Package (SPSS 16.0) and the graphs were drawn by Excel software. Comparisons between the means were carried out by Duncan method (P < 0.05).

RESULTS

Stem elongation and leaves color change over time

Results revealed an acceptable correlations between the leaves color components and the stage of stem growth in all cultivars, even in white cultivar 'Polar Star' (Fig. 3 and 4.). Among the models fitted to the data, linear and exponential models appeared to be best fitted, both showing high positive relationship between the changes of leaf color and stem elongation over time. In linear model, except for 'Polar Star' which showed the lowest correlation coefficient (R^2=0.89),

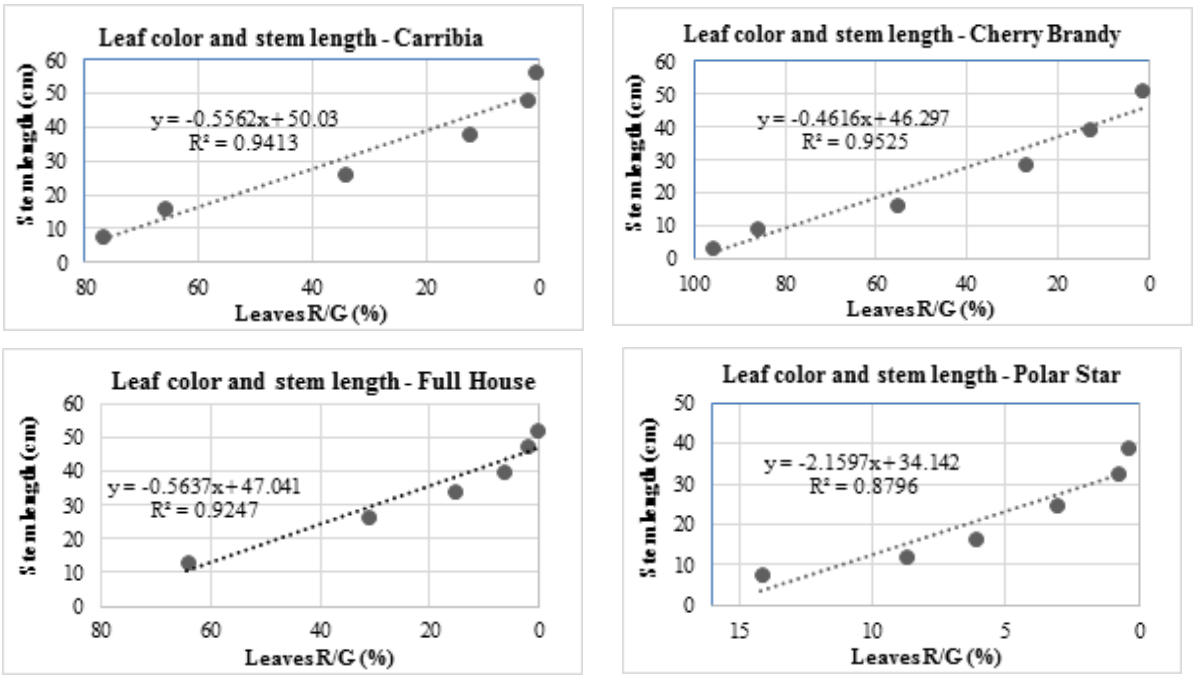

Fig. 3. Trend line of the relationship between stem length and leaves color (Linear model).

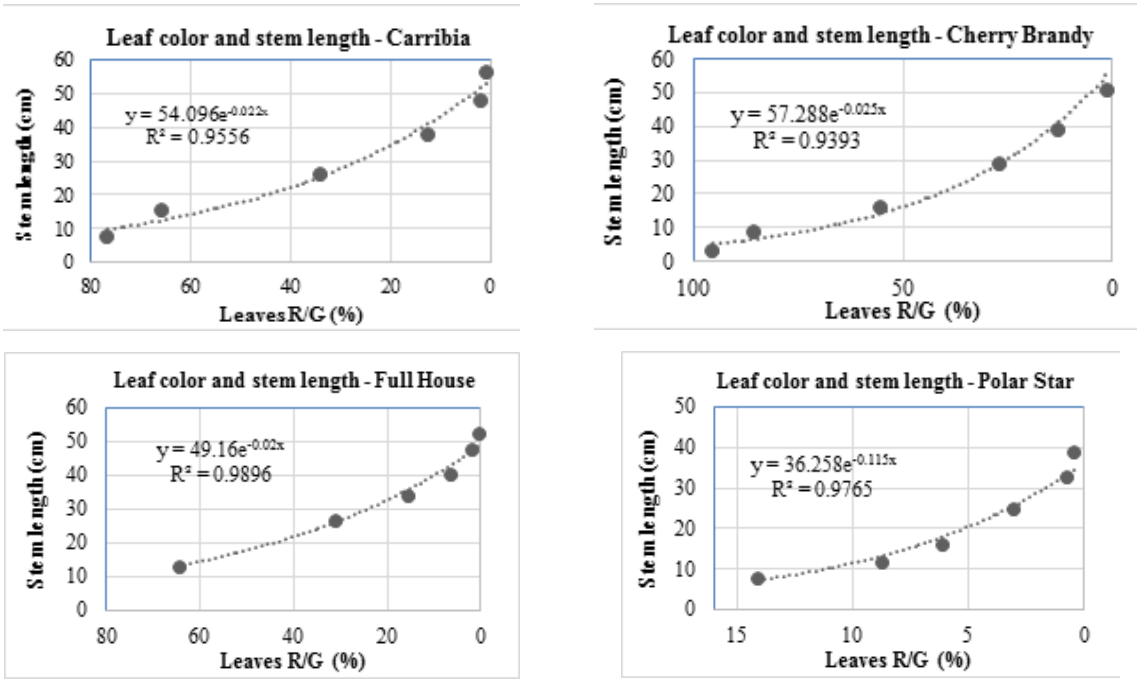

Fig. 4. Trend line of the relationship between stem length and leaves color (Exponential model).

other cultivars resulted in roughly similar coefficients. However, for exponential model, the correlation coefficients between the color components and the stem length were obtained 0.939 for 'Cherry Brandy', 0.955 for 'Carribia', 0.989 for 'Full House', and 0.976 for 'Polar Star' (Fig. 4). We also found that the model which can be best fitted to the data and describes the stem color-length relation precisely differs between the white and colorful cultivars (Fig. 5). In this case, quadratic model was fitted to the white cultivar, while linear model appeared to be the best choice for the colorful roses.

Stem layers and variations in leaf color components

The acquired data were undergone the ANOVA processes and the results were then presented in Table 3. As shown in this table, most color characteristics varied significantly from cul-

Table 3. Comparison of characteristics means among the cultivars.

	Chlorophyll	Carotenoid	SPAD	R	G	B	R+G	R+B	R+G+B
Top layer									
Caribia	72.21 b	22.5 b	41 b	206 b	207.3 b	202.4 bc	413 b	413.4 b	408.5 bc
Polar Star	96.93 a	17 c	64 a	220.3 a	222 a	216.8 a	442 a	442.3 a	437.1 a
Cherry Brandy	64.26 c	24 a	34.6 c	202.1 b	197 c	194.6 c	399 b	399.1 b	396.8 c
Full House	93.6 a	8.4 d	61.1 a	216.7 a	218 a	211.8 ab	435 a	435.2 a	428.5 ab
Middle layer	150 c								
Caribia		13.1 a	83.6 b	209.4 b	209.9 b	202.8 b	419 b	419.3 b	412.2 b
Polar Star	165.6 b	11.8 b	89.1 b	216.4 a	218.6 a	213.9 a	435 a	435 a	430.4 a
Cherry Brandy	133.7 d	12.1 ab	68.3 c	206.7 b	204.7 bc	199.9 b	411 b	411.4 b	406.6 bc
Full House	199.2 a	3.2 c	96.2 a	198.7 c	199 c	194.5 b	397 c	397.8 c	393.3 c
Bottom layer	141.9 a								
Caribia		11.2 a	71.6 a	212.1 a	210.8 ab	209.2 a	422 a	422.9 a	421.3 a
Polar Star	142.3 a	6.7 c	72.5 a	209.5 ab	212 a	204.5 ab	421 a	421.5 a	414 ab
Cherry Brandy	106.9 b	9.5 b	62.4 b	199.4 b	202 b	195.2 b	401 b	401.5 b	394.6 b
Full House	145.1 d	2.3 d	71 a	216.7 a	217.4 a	212.4 a	434 a	434 a	429.2 a

*Different letters show significant difference across the cultivars within the layers.

tivar to cultivar (Table 1). Although, the pattern of differences was not similar within each layer, cultivars showed different concentrations of chlorophyll, carotenoids and varied color components in each layer. The relationship between the SPAD readings and the total chlorophyll content of the leaves originated from the three different stem layers, which was directly measured by spectrophotometer, is presented in Fig. 6. As shown in this figure, there is a good correlation between the

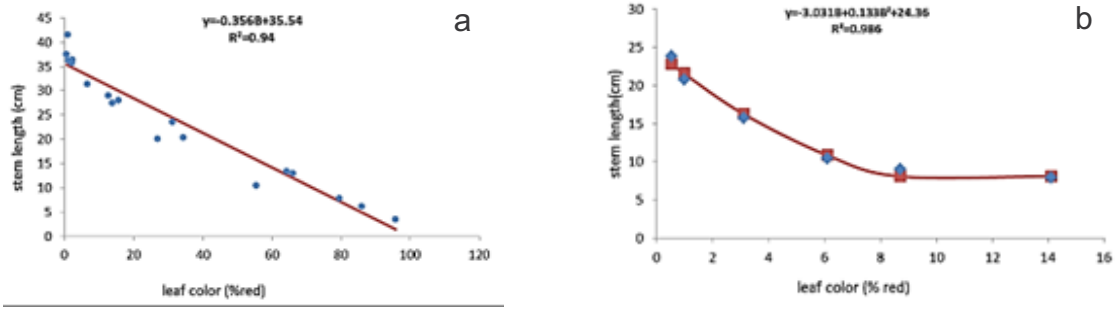

Fig. 5. Linear regression between stem length and red color count in colorful rose cultivars (a) and exponential regression in white color cultivar (b). (P < 0.05).

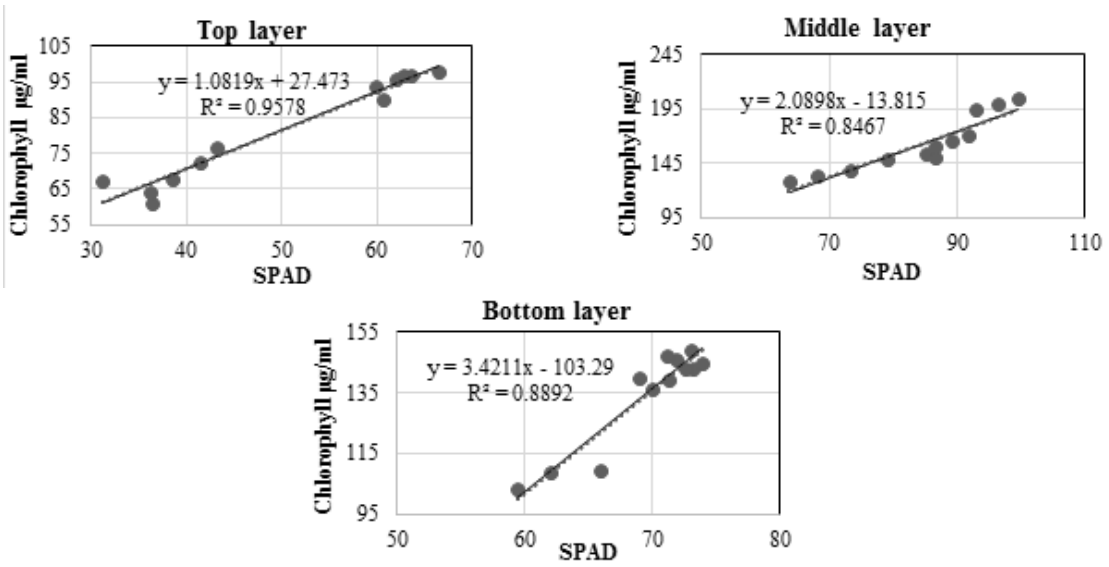

Fig. 6. Correlations between SPAD readings and direct measured leaf total chlorophyll content in three layers of rose stem.

SPAD readings and the direct-measured chlorophyll irrespective of stem layers. However, the correlation coefficient for top layer appeared to be slightly higher when compared with the other two layers. Despite of the significant difference between carotenoid concentrations among the cultivars, the correlation coefficient between this trait and the SPAD readings was less than 0.8. Such correlation between SPAD and the other color components such as R, G, and B was found to be even much lower. Comparison of the layers by their chlorophyll contents indicated that this compound is much abundant in the layers situated at the middle and bottom than the layer at the top. The cultivar 'Polar Star' which produces white flowers was enriched in chlorophyll and showed the highest amount of this material in the top layer, while conversely, the colorful cultivar 'Cherry Brandy' was highly provided with carotenoid compounds, hence leaving poor chlorophyll contend behind instead (Table 3). Another important finding from Table 3 is that comparing the top layer with the bottom layer, one can understand that the bottom layer is highly enriched in chlorophyll, considering chl/cart. ratio, while this ratio is lower for the top layer.

DISCUSSION

Color in image analysis is a powerful descriptor which facilitates recognition and detection of objects. In this study, image analysis indicated its capability in detection of color differences among the leaves growing on different cultivars over the entire period of stem development. Additionally, a good correlation was found between the stem leaf color change and stem length, showing that pigments alteration inside the leaves obey a trend which is very similar to the stem growth pattern. Anthocyanins beside the other most important pigments such as chlorophylls and carotenoids compose a dominant part of the pigment pools inside the leaves and flowers. There are many internal and external factors affecting pigment concentration and distribution across the rose plant, especially leaves and flowers (Schmitzer *et al.,* 2009). In most rose cultivars, young shoots usually are warm-colored especially in bronze hues and they gradually loss their colorful pigments by shoot growth and start to develop chlorophyll which is the most important pigment involved in the photosynthesis process. This gradual change in leaf color is in harmony with the shoot stage of growth and can give cut-rose growers an opportunity not only to plan the greenhouse operational programs but also to adjust the harvest time and schedule yearly cropping period. In 'Asami' rose, image analysis was used to estimate leaf area coverage and to build a relationship between the LAI and the plant ground occupied area (Shimomura *et al*., 2003). It has been also found that image analysis has the ability of detecting some visual differences among turf grass varieties, helping researchers to classify them according to their aesthetic appeal and qualities (Leinauer and Sevostianova, 2014). Image analysis was used for precise tracking and quantification of dicot leaves' growth with a time resolution of minutes and a spatial resolution of a few millimeters (Schmundt *et al.*, 1998). Among the models tested, it was appeared that the exponential model did slightly better, at least in some cultivars, than the linear model in predicting instantaneous stem height. It was shown that non-linear model can be used to interpret the relationship between the plant growth and the crop entropy by means of image analysis technique (Asefpour and Massah, 2012). In colorimetric studying of some agricultural fruits, exponential and linear models were fitted to data acquired before and after the transformation of the RGB values, respectively (Mendoza *et al*., 2013). Our results also suggested the possibility of finding a reasonable correlation between the foliage color variations over time and the cut-rose stem maturation phases in order to provide the growers with a tool to assist them in predicting the crop growth stages at a greenhouse scale.

Significant differences observed in most color components of leaves within each layer of different cultivars imply the existence of different concentrations and varied distributions of pigments throughout the foliage. The content of leaf chlorophyll is an important indicator of the growth and physiological status of a plant, and is directly related to photosynthetic potential and primary production (Curran *et al.*, 1990). Additionally, leaf chlorophyll amounts are directly affected by

plant stress and senescence (Hendry *et al.*, 1987). Chlorophyll may be used as an indirect indicator of nitrogen levels (Amaliotis *et al*., 2004). Nitrogen content in the leaf is in relation with the color of leaves (Cabrera, 2004). Therefore, estimation of chlorophyll may be carried out to determine the nitrogen content in the plant, rather than the plant growth itself. We saw leaf chlorophyll content exhibited a relationship with the growth of the plant. The traditional method to chlorophyll measurement is a laboratory method that measures foliar chlorophyll concentration by pigment extraction in a solvent and spectrophotometric analysis. It requires destructive sampling and is time consuming (Porra *et al*., 1989). Digital processing of images has been used with success in crop management (Pydipati *et al.*, 2006).

The results of the present study showed that the image analysis could be a suitable approach for monitoring plant growth status. Our results clearly indicated that the stem height in cut roses is in close harmony with the leaf color. This work is a starting point for the development of a model relating the plant visual dynamic variations to the leaves color variations. A similar technique was developed and introduced by Lanaa *et al*. (2006) who used digitized images to estimate fruit ripening by means of RGB color system analysis. The simple models introduced in this study may be subjected to some generalizations by including the behavior of as many cultivars as possible in order to be integrated in future complete plant visual monitoring models possibly programmed in plant growth software to assist greenhouse rose growers. We showed that image analysis method was faster and possibly easier to use for recognizing growth level in cut roses as compared to human visual judgment. This method can be used to perform wise management and make much more economical and optimal decisions for large scale greenhouse production systems.

CONCLUSION

In conclusion, we presented a non-destructive method for estimating stem developmental stages in some cultivars of cut rose plants. The estimated models were capable to make a link between the stem leaf color status and the stem length during almost whole growing period of cut roses. On the other hand, image analysis proved to be much efficient in determining leaf color variations not only according to the shoot growth stages but also based on the leaf age classes within the stem layers. Moreover, it was concluded that the leaf color variations by plant growth which normally happen in most garden and greenhouse roses could be used as a distinctive characteristic in predicting plant morphological changes in rose plants and of course, in other plants with the similar apparent color variations during the growth period.

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