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Comparing digital image analysis and visual rating of gamma ray induced Bent grass (*Agrostis stolonifera*) mutants

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ABSTRACT

The effectiveness of digital image analysis (DIA) for determining turf quality over visual rating was judged. It provides an alternative method to measure the reflectance from vegetative surfaces and showed strong agreement with visual ratings in evaluating turf color. It is clear from the data that the correlations of hue and dark green colour index (DGCI) were significantly positive with the parameters of visual rating. There were non-significant correlation of brightness with quality and texture, and saturation and texture. The DGCI values were in line with each of these parameters when the slope of regression line was significantly different from zero (P<0.05). These relationships were better in DGCI and quality; DGCI and colour and DGCI and texture. Non-linear relationship was noticed between DGCI and saturation and DGCI and brightness. Therefore, digital photography and subsequent image analysis was capable of quantifying turf grass color in field experiments.

Key words: *Agrostis stolonifera*, Digital image analysis, Dark green color index, Turf quality

Spectral reflectance analysis (digital image analysis) has been introduced as an alternative to visual ratings for assessment of turf quality as a quick, reliable, and non-destructive methods (Da Costa *et al*. 2004). Canopy spectral reflectance measurements have been used to estimate plant quality under different irrigation and/or fertilization applications (Baghzouz *et al*. 2007).

Digital Image Analysis (DIA) provides an alternative method to measure the reflectance from vegetative surfaces. Tiwari *et al*. (2014) found that DIA showed strong agreement with visual ratings in evaluating turf color. DIA provides an objective, unbiased, non-destructive and consistent measurements. This technique provides rapid, accurate, and precise results as recent digital image collection equipment and image analysis software have the capability to acquire and process hundreds of images per hour and images can be stored for further analysis at the researcher's convenience (Díaz-Lago *et al*. 2003). Digital imagery process is also a cost-effective technique as it requires only a digital camera, computer, and an image analysis program. A low-cost digital camera, with white balance adjusting, is sufficient for collecting images with low-quality Joint Photographers Expert Group (JPEG) compression format. Tiwari *et al*. (2014) concluded that results from digital image analyses, using low-quality (JPEG) images, have a number of desirable qualities for data quantification and have the same results of those of a loss less format such as TIFF format or RAW images. Therefore, digital photography and subsequent image analysis may be capable of quantifying turf grass color in field experiments. The objective of this study was to rapidly generate variability through mutagenesis and quantifying the differences in quality of irradiated *Agrostis stolonifera* L. population by using digital camera image analysis and supported by the software using a hue saturation and brightness (HSB) color scale.

MATERIALS AND METHODS

Irradiation with gamma rays of *Agrostis stolonifera* with CO_{60} was done on 30 uniform stolon (sprigs) set of propagules for each treatment. These were irradiated with nine doses (2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5, 20.0, 22.50 KRD) of gamma rays at national physical laboratories, Indian Agricultural Research Institute, New Delhi during October 2012, subsequently the mutants were named as T_1 to T_9, respectively. A set of 30 untreated stolons was used as control. Each treated sprig (propagule of grass) was planted in a pot and further clonally multiplied. After multiplication these were planted in 3 × 2 meter beds with three replications of each treatment.

As per the rating of NTEP, each treatment was visually rated for color, texture and overall quality throughout the growing season using 1 to 9 scale by 5 evaluators, where 9 represents ideal dark green, uniform colour; 6 represents acceptable; and 1 represents unacceptable yellow/brown

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colour of turf. Texture was visually rated on 1 to 9 scale, where 9 represents extremely fine-texture (narrow leaf blade), 5 represent moderately fine and 1 represents very coarse texture unacceptable (wide leaf blade). Similarly, overall quality was evaluated.

Turf quality was evaluated by using digital image analysis process that include;(1) acquiring digital images by a digital camera in jpeg (joint photographic experts group, .jpg) format under consistent lighting,(2) extracting the red, green and blue (RGB) levels for all pixels in the acquired images using Image software, (3)converting the RGB levels into Hue, Saturation and Brightness (HSB) and (4) creating a turf color index from the HSB values known as the dark green color index (Eq.2) (Karcher and Richardson 2003, Tiwari *et al.* 2014).

$$DGCI = [(H-60)/60 + (1–S)+(1–B)]/3 \quad (2)$$

where, DGCI = dark green color index, H, S, B=hue, saturation, and brightness levels.

All digital images in these studies were taken with a CANON EOS 60D camera. The images were collected in JPEG format, with a color depth of 16.7 million colors, and an image size of 640×480 pixels (about 80 kilobytes per image). Camera settings were adjusted manually to ensure the same conditions for all images and were set to a shutter speed of 1/8 s, an aperture setting of, F 2.8, and a focal length of 80 mm. All images were captured using a uniform light source to prevent any changes in light source due to shadows or cloudy weather. The camera was adjusted manually for white balance by using a grey piece of paper to adjust the camera's color sensitivity to preserve natural colors under the fluorescent lighting inside the box (Karcher and Richardson, 2003).

The plots were photographed on November 2013 in between 13.25 and 13.35 h during overcast conditions (illuminance ˜ 5000lux).Calibrations of images were taken in dark conditions using only the camera flash as a light source. Digital Images were taken on each replication of nine treatments (2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5, 20.0, 22.50 KRD) along with untreated control. These were transferred to a personal computer and analysed for HSB levels using the methods described by Karcher and Richardson (2003). One-way ANOVA was performed using PROC GLM in SAS Statistical Software on the HSB and DGCI data sets, with treatment as the variable. For a given parameter, differences were determined significant among treatments when the ANOVA f test had a corresponding P value 0.05. In such cases, a Fisher's protected LSD test was performed to separate treatments' differences (Freund and Wilson 1993). Correlation coefficients and linear regression analysis were used to judge the performance of DGCI taken as dependable variable. The Pearson's correlation coefficients (*r*) were determined by constructing a correlation matrix between visual rating and DGCI using the PROCCORR procedure of the Statistical Analysis System (9.1 edition, SAS Institute, Cary, NC) using all data set for years 2012, and 2013. Linear regression analyses were conducted for all turf quality and data collected across treatments and replications to determine the relationships between different turf quality indices and DGCI developed by the digital imagery analysis process.

RESULTS AND DISCUSSION

Differences in turf colour and quality as recorded by mutants following visual and digital image analysis were quantified and the descriptive statistics is represented in Table 1.All five evaluators observed differences in color and quality of the mutants. Based on the turf color and quality of mutant T_9 followed by mutant T_8 were superior over others. The data indicated that the coefficient of variation (CV %) was higher for all the visual rating parameters, i e quality, color and texture. The lowest values of coefficient of variation were recorded in DGCI (1.06 %) and hue (1.12%).There were significant differences among mutants with regard to HSB and DGCI. Amongst mutants, T_9 had maximum hue followed by the mutant T_8.

Table 1 Anova of various parameter as affected by treatments

Traetment	Quality	Colour	Texture	Hue	Saturation	Brightnes	DGCI
t0	6.0ab	5.8a	6.0ab	32.7c	61.84b	186.0c	0.45c
t1	6.0ab	5.2ab	5.4b	32.94bc	52.03c	178.6c	0.4502ab
t2	6.4ab	6.4ab	6.8a	32.74c	60.24b	197.0a	0.4524ac
t3	6.4ab	6.0ab	6.8a	32.94bc	50.28c	189.2ab	0.4524ac
t4	5.0b	6.8b	6.8a	32.77c	57.84c	196.4a	0.4518b
t5	5.6ab	6.2b	5.4b	32.9c	72.54a	175.0c	0.4498b
t6	5.6 ab	6.4b	5.2b	33bc	70.24a	193.4b	0.4506ac
t7	5.8ab	6.4b	6.2ab	32.96bc	74.63a	173.8ab	0.4516ab
t8	6.8 a	7.4c	7.0a	33.4b	64.24ab	193.8ab	0.456ab
t9	7.0a	8.6d	7.0a	34.28a	73.41a	205.4a	0.4562a
Mean	6.06	6.52	6.26	33.06	63.73075	188.86	0.4521
F Value	1.34*	8.23**	2.32*	8.06**	9.82**	2.89**	1.17**
Coeff Var(%)	19.244	11.165	16.830	1.12	9.882	7.249	1.057

Table 2 Correlation Analysis among visual rating and DIA

	Quality	Colour	Texture
Hue	0.28377*	0.47729*	0.25046*
Saturation	0.00375*	0.39676*	–0.09939
Brightness	0.09447	0.49325*	0.23051
DGCI	0.11786**	0.33076**	0.15282**

*Significant at 5 %, ** significant at 1 %

The correlations amongst DIA (HSB, DGCI) and NETP visual ratings are presented in Table 2. It is clear from the data that the correlations of hue and DGCI were significantly positive with all the parameters of visual rating at 5 % and 1% level of significance respectively. There was non-significant correlation of brightness with quality and texture, saturation and texture.

Six separate linear regression analysis were performed using Proc REG in SAS statistical software (SAS Institute; 1996). The DGCI values were analyzed as the dependable variable and quality, colour, texture (Visual rating) and HSB as independable variables. The DGCI values were in tune with each of these parameters when the slope of regression line was significantly different from zero ($P<0.05$) (Freund and Wilson 1993) These relationships were better in DGCI and quality ($r^2=0.55$); DGCI and colour ($r^2=0.7363$) and DGCI and texture ($r^2=0.627$). Non linear relationship was noticed between DGCI and saturation ($r^2=0.0011$) and DGCI and brightness ($r^2=0.0007$).

The significantly greater CV% with visual ratings (Table 1) suggests that rating values are evaluator dependent and that evaluators are likely to vary in ranking different shades of green (Goodenough and Goodenough 2012). The higher rate of survival in lower doses and poor survival at higher doses were also recorded in gladiolus (Tiwari *et al*. 2010) and calendula (Tiwari and Kumar 2011) plants. Differences in assessment by human occur because individual differs in his capability to perceive various wave lengths of visible light, which can lead to differences in estimates of turf quality (Tiwari *et al.* 2014). This visual rating scale is mainly a function of color, density, and uniformity (Karcher and Richardson 2003). This rating system is biased due to subjectivities of the raters and has inaccurate estimation of turf quality (Tiwari *et al*. 2014). Among HSB, hue has been found to be the best indicator of the visual color of the turf (Karcher and Richardson 2003). These differences in color are in strong agreement with results of visual rating where mutants' T_8 and T_9 were significantly different than parent. Significant differences in DGCI were also observed among T_8, T_9 and parents. This may be due to genetic changes in mutants.

The ability to distinguish color differences among turf variants as H, S, or B differences is a significant advantage of digital image analysis over subjective visual ratings. Comparing both visual ratings and digital image analysis, the statistical rankings of treatment means were similar between the two methods. As the DGCI coefficient of variance (Table 1) was significantly lower than rater visual parameter. The DGCI is a more consistent measure of dark green color across mutants than the visual parameters individual DIA measurements of H, S, or B. DIA provides an objective, unbiased, nondestructive, and consistent measurements. This technique is capable of providing rapid, accurate, and precise results as recent digital image collection equipment and image analysis software have the capability to acquire and process hundreds of images per hour and images can be stored for further analysis at the researcher's convenience (Díaz-Lago *et al*. 2003). Digital imagery process is a cost-effective technique as it requires only a digital camera, computer and an image analysis program. The importance of use of digital image analysis for measurement of turf color has already been discussed by Karcher and Richardson (2003).

CONCLUSION

Digital image analysis provides objective, quantitative turf quality estimate and little to no prior experience is needed. On the other hand, visual rating techniques need substantial training and measurements may vary because of its subjectivity and inherent error in human evaluators. In contrast to visual ratings DGCI of DIA are recorded on a continuous scale which brings turf quality estimates to more realistic. A digital image of mutants, varied in visual color due to genetically controlled differences, was quantified by digital image analysis and visual rating. It is demonstrated that digital image analysis is a suitable tool to assess turf grass colour in comparison to visual rating.

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