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Comparing digital image analysis and visual rating of gamma ray induced Kentucky bluegrass *(Poa pratensis)* mutants

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ABSTRACT

Variability was generated in Kentucky bluegrass (*Poa pratensis*) through gamma-ray irradiation and genotypes were evaluated for their response to low management, induction of dwarfness and other quality attributes. The main objective of this study was to judge the suitability of digital image analysis over visual rating of turf quality and to identify changes in mutants, and correlations among visual rating and digital image analysis were computed. Differences were significant among mutants with respect to hue, brightness and saturation. Significant and positive correlations of hue and DGCI were observed with all the parameters of visual rating. There were non-significant correlations of brightness with quality, brightness with texture, saturation and texture. These relationships were better in DGCI and color (r^2=0.123) DGCI and brightness (r^2=0.0849); DGCI and hue (r^2=0.0772) and DGCI texture (r^2=0.0325). Non-linear relationship was noticed between DGCI and saturation (r^2=0.0011).

Key words: Dark green color index, Dark image analysis, Digital image analysis, *Poa pratensis*

Mutation is an important tool for breeding new cultivars and a large number of cultivars have been bred through spontaneous or induced mutations. Gamma rays are widely used for mutation induction in plants. Precise and quick evaluation of mutants strengthens the crop improvement programme. Traditional methods of determining turf quality have been based on a visual rating system as per the National Turfgrass Evaluation Program (NTEP) with a scale ranging from 1 to 9, with turf quality increasing with increased rating. A rating of 5 or less represents inferior quality and is unacceptable (Morris 2002). This scale is mainly a function of color, density, and uniformity (Tiwari *et al*. 2014a). Differences in human assessment occur because of differences in individual's capability to perceive wavelengths of visible light (Mirik *et al.* 2006 Tiwari *et al*. 2014b) therefore, NTEP rating system may be erroneous due to subjectivities of the raters (Keskin *et al.* 2003). The visual assessments are fast and easy to perform (Stafford *et al.* 2013). Turf color is the key component of aesthetic quality and a good indicator of grass health. Timely quantification of turfgrass colour that uses readily accessible equipment would strengthen the validity of study results without adding significant burden to the evaluation process. Several techniques such as reflectance measurements, chlorophyll and amino acid analysis and comparison with standard colours have been used to objectively measure turf colour. However all of these methods have certain disadvantages compared with subjective colour ratings. Reflectance chlorophyll and amino acid measurements require expensive equipments and transport of samples to a laboratory for analysis. Also the correlations between colour and chlorophyll or amino acid measurements are either species or cultivar dependant. Though the use of standardized colour chart is effective but the results are not possible to analyse statistically. The shortcoming with the colorimeter studies is the relatively small (<20cm^2) measurement area which require numerous sub sample measurements to represent typical turfgrass plots. 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 method to measure the reflectance from vegetative surfaces (Da Costa *et al.* 2004, Karcher and Richardson 2003).

Karcher and Richardson (2003) found that the Digital Image Analysis (DIA) showed strong agreement with visual rating in evaluating turf color. They developed dark green colour index (DGCI) by using hue, saturation, and brightness (HSB) levels. DIA provides, an objective, unbiased, non destructive and repeatable measurement. This technique provides rapid, accurate, and precise results as recent digital camera and image analysis software have the capability to acquire and process hundreds of image in short period and

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Table 1 Anova of various parameters as affected by various treatments

Treatment	Quality	Colour	Texture	Hue	Saturation	Brightness	DGCI
T0	7 ab	6.8 ab	6.6 b	31.7 d	61.96 c	186 b	0.45274 b
T1	7 ab	6.2 b	6.8 bc	33.34 ab	52.17 d	180.8 b	0.45 ab
T2	7.4 ab	7.4 ab	7.8 a	34.54 a	60.44 c	197 ab	0.4522 ab
T3	7.4 ab	7.0 ab	7.6 ab	33.74 b	50.56 d	189.8 b	0.4544 ab
T4	7 ab	7.8 ab	7.0 abc	34.77 a	57.72 c	196.8 ab	0.4524 ab
T5	6.6 ab	7.2 ab	5.6 c	33.9 b	72.84 a	175.6 b	0.4516 b
T6	6.2 b	7 .0 ab	5.8 c	33.26 c	70.24 b	194 ab	0.4502 b
T7	6.2 b	7.0 ab	7.0 a	33.44 c	74.77 a	174.8 b	0.4508 ab
T8	7.5 a	7.8 ab	7.0 a	33.86 b	64.88 bc	196.4 ab	0.4518 a
T9	7.6 a	8.6 a	7.0 a	34.3 ab	73.93 a	209.6 a	0.4568 a
Mean	6.94	7.28	6.82	33.68423	63.95174	190.08	0.45274
F Value	1.04*	4.75**	4.32**	17.96**	10.59**	3.25 *	1.72 *
Coeff Var(%)	15.18106	9.316387	10.87419	1.347844	8.540716	7.128917	0.968088

*Means with the same letter are not significantly different

image can be stored for further analysis at the researcher's convenience (Diaz-Lago *et al.* 2003 and Tiwari *et al.* 2010)) and is cost effective. A low-cost digital camera, with white balance adjusting, is sufficient for collecting images with low-quality Joint Photographers Expert Group (JPEG) compression format. It has a number of desirable qualities for data quantification and have the same results as those of a loss less format such as TIFF or RAW images (Steddom *et al.* 2004). Therefore, DIA may be capable of quantifying turf color in field experiments. The objective of this study was to generate variability through mutagenesis and quantifying the differences in quality of irradiated Kentucky bluegrass population by use of digital camera image analysis and supported by the software using a HSB colour scale.

MATERIALS AND METHODS

Kentucky bluegrass was subjected to gamma rays irradiation from CO_{60} source. Thirty uniform stolon (sprigs) sets of propagules were irradiated with nine doses (5.0, 7.5, 10.0, 12.5, 15.0, 17.5, 20.0, 22.50, 25.00 Gy) of gamma rays at National Physical Laboratory, Indian Agricultural Research Institute, New Delhi during October 2012. A set of 30 untreated stolon were used as control. Each treated sprig was planted in a pot and further multiplied clonally. After multiplication these were planted in 3.0m x 2.0m bed with three replications of each treatment and named as T_0 to T_9 where T_0 represented control genotype.

Each treatment was visually rated for colour, texture and overall quality (Morris 2002) throughout the growing season by 5 evaluators using 1 to 9 scale where 9 represents ideal dark green, uniform colour; 6 represents acceptable; and 1 represents unacceptable yellow/brown colour of turf. Similarly 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 unacceptable texture (wide leaf blade). The overall quality was also evaluated on 1 to 9 scale where 9 representing superior quality and 1 representing unacceptable quality.

Digital image analysis process included; (1) capturing digital image by a digital camera, (2) extracting the red, green and blue (RGB) levels for all pixels in the acquired images using Image software (Sigma scan Pro 5), (3) converting the RGB levels into Hue, Saturation and Brightness (HSB) and (4) creating a color index known as the dark green color index from the HSB values as reported by Karcher and Richardson (2003).

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

The digital images were taken with a CANON EOS 60 D camera. The images were collected in JPEG format, under consistent uniform light source (Ikemura, 2003) 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 colours under the fluorescent lighting inside the box. These were collected in JPEG format with a colour depth of 16.7 million colours, and an image size of 640x480 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.

The plots were photographed during November 2013 in between 13.25 and 13.35 h during overcast conditions (illuminanceH"5000 lux). Calibrations of camera settings were done in dark conditions using only the camera flash as a light source. The images were taken and transferred to a desk top 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 treatments as a variable. An F-test was used to test for significant differences in variance between DIA and other analysis methods. Correlation coefficients and linear regression analysis were used to determine the relationship between different turf quality indices and DGCI developed by the digital imagery analysis process. The Pearson's correlation coefficients (r) were determined by constructing

Table 2 Pearson correlation statistics (Fisher's z transformation)

Variable	With Variable	N	Sample correlation	Fisher's z	Bias adjustment	Correlation estimate	95% confidence	limits	(H0: rho=rho0) rho0	p value
Quality	Colour	50	0.128	0.129	0.001	0.127	–0.157	0.391	0	0.378
Quality	Texture	50	0.030	0.030	0.000	0.030	–0.251	0.305	0	0.837
Quality	Hue	50	0.067	0.067	0.001	0.066	–0.216	0.338	0	0.647
Quality	Saturation	50	–0.201	–0.204	–0.002	–0.199	–0.452	0.084	0	0.162
Quality	Brightness	50	0.152	0.154	0.002	0.151	–0.133	0.412	0	0.293
Quality	DGCI	50	0.117	0.117	0.001	0.116	–0.168	0.382	0	0.421
Colour	Texture	50	–0.012	–0.012	0.000	–0.012	–0.289	0.268	0	0.936
Colour	Hue	50	0.344	0.358	0.004	0.341	0.069	0.565	0	0.014
Colour	Saturation	50	0.314	0.325	0.003	0.311	0.036	0.542	0	0.026
Colour	Brightness	50	0.481	0.524	0.005	0.477	0.230	0.667	0	0.000
Colour	DGCI	50	0.351	0.366	0.004	0.348	0.077	0.571	0	
Texture	Hue	50	0.278	0.285	0.003	0.275	–0.003	0.514	0	0.051
Texture	Saturation	50	–0.319	–0.330	–0.003	–0.316	–0.546	–0.041	0	0.024
Texture	Brightness	50	0.128	0.128	0.001	0.126	–0.158	0.391	0	0.380
Texture	DGCI	50	0.194	0.196	0.002	0.192	–0.091	0.446	0	0.179
Hue	Saturation	50	–0.009	–0.009	0.000	–0.008	–0.286	0.271	0	0.954
Hue	Brightness	50	0.274	0.281	0.003	0.271	–0.008	0.511	0	0.054
Hue	DGCI	50	0.278	0.285	0.003	0.275	–0.003	0.514	0	0.050
Saturation	Brightness	50	0.239	0.244	0.002	0.237	–0.044	0.483	0	0.094
Saturation	DGCI	50	0.101	0.101	0.001	0.100	–0.184	0.368	0	0.489
Brightness	DGCI	50	0.291	0.300	0.003	0.289	0.011	0.525	0	0.040

a correlation matrix between visual rating and DGCI using the PROCCORR procedure for the Statistical Analysis system (9.1 edition; SAS Institute, Cary, NC) using all data set for years 2012, and 2013.

RESULTS AND DISCUSSION

Differences in turf colour and quality as recorded by mutants following visual and digital image analysis were quantified and represented in Table 1. All five evaluators observed differences in color and quality of the mutants. Based on the turf color and quality, 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 (0.968%) followed by hue (1.34%). There were significant differences among mutants with regard to HSB and DGCI. Amongst mutants, mutant t_4 had maximum hue followed by the mutant t_2.

The Pearson correlation statistics along with Fisher's Z transformation amongst DIA (HSB, DGCI) and NETP visual rating are given 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% level of significance. There were non-significant correlations of brightness with quality and texture, and saturation and texture.

Six separate linear regression analyses were performed using Proc REG in SAS statistical software (SAS Institute; 1996). The DGCI values were analyzed as the dependable variable and quality, color, texture and HSB as in dependable 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 color (r^2=0.123) DGCI and brightness (r^2=0.0849); DGCI and hue (r^2=0.0772) and DGCI and texture (r^2=0.0325). Non-linear relationship was noticed between DGCI and saturation (r^2=0.0011).

The significantly greater CV% with visual ratings (Table 1) suggests that rating values were evaluator dependent and that evaluators are likely to vary in ranking different shades of green (Goodenough and Goodenough, 2012 and Tiwari *et al*. 2015). Differences in assessment occur because individual differs in his capability to perceive various wavelengths of visible light, which lead to differences in estimates of turf quality (Miriket *et al.* 2006). This visual rating scale is mainly a function of color, density, and uniformity (Newton 2007). This rating system is biased due to subjectivities of the raters and has inaccurate estimation of turf quality (Keskin *et al.* 2003). Among HSB, hue has been found to be the best indicator of the visual color of the turf (Stafford *et al.* 2013). These differences in color are in strong agreement with results of visual rating where mutants t_8 and t_9 significantly different 6 than the parent. Significant differences in DGCI were also observed among t_8, t_9 and parent. This may be due to genetic changes in mutants. The ability to distinguish color differences among turf variants as H, S, or B difference is a significant advantage of digital image analysis over subjective visual ratings. The importance of use of digital image analysis for measurement of turf color has already been discussed by Karcher and Richardson

(2003).

Comparing both visual ratings and digital image analysis, the statistical ranking of treatment means was similar between the two methods. As the DGCI coefficient of variance (Table 1) was significantly lower than rater visual parameters. The DGCI is a more consistent measure of dark green across mutants than the visual parameters individual DIA measurement of H, S, or B. DIA provides an objective, unbiased, non-destructive, and consistent measurement. 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 (Diaz-Lago *et al.*, 2003). The digital imagery process is a cost-effective technique as it requires only a single person, a digital camera, computer, and an image analysis program.

The evaluation and comparison of two turf quality evaluation techniques that were considered in this study enabled us to draw the following conclusion about the digital imagery process. Digital image analysis provides objective, quantitative turf quality evaluation and little or no prior experience is needed. On the other hand, visual rating technique needs substantial training and measurement may vary from day to day for the same evaluator and different values may be reported because of its subjectivity and inherent error in human evaluators. Visual ratings are recorded on a discrete scale, but DGCI values are recorded on a continuous scale which brings turf quality estimates to be realistic. DIA is simple, core effective, fast and can be performed by a single individual. A digital image of various mutants, varied in visual color due to genetically controlled differences which was quantified by digital image analysis and visual rating. It is demonstrated that image analysis is a suitable tool to assess turfgrass colour.

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