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Mutagenesis and digital image analysis of mutants for quality attributes of native *Cynodon dactylon*

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ABSTRACT

The ability to capture information of turf grass *in situ* makes digital camera based image analysis, a viable tool to quantify turf grass (*Cynodon dactylon* Pers.) in field experiments. In addition to colour quantification, digital image analysis has been used successfully to quantify percentage turf grass cover and has also been proved to be useful in quantifying turf parameters such as weed infestation, disease incidence, herbicide toxicity, leaf area and recovery from injury. Colour is one of the major criteria used to evaluate the quality of turf and lawn. To generate variability in Bermuda grass to select genotypes responsive to low management, gamma-ray irradiation was used for induction of dwarfness and other quality attributes. Five dwarf mutant lines (DFR 440, DFR-C-444, DFR-C-445, DFR-C 446 and DFR-C-448) were isolated. In the present study, camera and image analysis technique is applied to measure turf colour by its reflectance in the HSB colour scale. The data depicts that the dwarf mutant lines had better quality of lower canopy height, shorter internodes and shorter leaves than the parent. It is demonstrated that image analysis is a suitable non-destructive tool to assess turf grass colour in a reproducible and calibrated manner, over a wide span of structural and colour attributes of turf grass.

Key words: *Cynodon dactylon*, Colour, Image processing, Turf grass

Cynodon dactylon Pers. is highly valued warm season turf grass having global adaptability, robustness and resistance to trampling. In India, it is widely used as turf grass, and is also effective in soil conservation. Conventional methods of determining turf quality have often been based on visual rating system as also developed by the National Turf grass Evaluation Program (NTEP), USA with a scale ranging from 1 to 9, representing the lowest quality to the highest quality. The rating results, however, are influenced by individuals, are subjective in nature and are difficult to reproduce. Other techniques used to objectively measure turf colour, including reflectance measurements (Camelo *et al*. 2012), chlorophyll and amino acid analysis (Laurinavicius *et al*. 2012), and comparison with standardized colors (Goodenough and Goodenough 2012) have certain disadvantages compared with subjective colour ratings. The use of standardized colour charts to measure turf colour is effective, but results are not possible to statistically analyze with traditional ANOVA techniques.

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 tool (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). Therefore, digital photography and subsequent image analysis may be capable of quantifying turf grass colour in field experiments. The objective of this study was to rapidly generate variability through mutagenesis and rapidly quantifying the differences in quality of *Cynodon dactylon* mutants by the use of digital camera image analysis and supported by the software using an hue, saturation and brightness (HSB) colour scale.

MATERIALS AND METHODS

Irradiation with gamma rays of *Cynodon dactylon* (local turf grass) with CO_{60} was done on 30 uniform stolen (sprigs) sets of propagules for each treatment. These were irradiated with nine doses (2.5, 5.0, 7.5, 10.0, 15.0, 20.0, 30.0, 35.0, 40.0 kr) of gamma rays at NPL, IARI during October 2012. A set of 30 untreated stolen was used as control. Each treated sprig was planted in a pot and further clonally multiplied. After multiplication these were planted in 3 m × 2 m beds with three replications of each treatment.

As per the rating of NTEP, each treatment was visually rated for colour, texture and growth habit throughout the

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growing season using 1 to 9 scale, where 9 represents ideal dark green, dense, uniform turf; 6 represents acceptable quality; and 1 represents yellow/brown/dead turf. Similarly texture was visually rated on a 1 to 9 scale, where 9 represents extremely fine-texture (narrow leaf blade) and 1 represents very coarse texture (wide leaf blade).

Millions of bits of information on a turf grass canopy can be obtained through digital photography from an image taken of a turf plot using a 1280 × 960 pixel resolution containing 1228 × 800 pixels, with each pixel containing independent colour information of the turf plot. The information contained in a digital image includes the amount of red, green and blue (RGB) light emitted for each pixel in the image. Although it may be intuitive to use the green levels of the RGB information to quantify the green colour of an image, the intensity of red and blue will confound how green an image appears. To ease the interpretation of digital colour data, RGB values are converted directly to HSB values that are based on human perception of colour. In HSB colour description, hue is defined as an angle on a continuous circular scale from 0 to 360° (0°=red, 60°=yellow, 120°=green, 180°=cyan, 240°=blue, 300°=magenta), saturation is the purity of the colour from 0% (gray) to 100% (fully saturated colour), and brightness is the relative lightness or darkness of the colour from 0% (black) to 100% (white) (Adobe Systems 2002).

The process used to determine the average colour of a digital image include: (i) acquiring an image with digital photography, (ii) obtaining the average RGB pixel levels for the image and (iii) converting the RGB levels to the more intuitive HSB parameters. All digital images in these studies were taken with a CANONEOS 60D camera. The images were collected in the JPEG (joint photographic experts group, .jpg) format, with a colour depth of 16.7 million colours, and an image size of 1280 × 960 pixels (≈ 260 kilobytes per image). Camera settings included a shutter speed of 1/400s, an aperture of 1/4.0, and a focal length of 32 mm. Images were transferred to a personal computer for subsequent analysis. The average RGB levels of the digital images were calculated using Sigma Scan Proversion 5.0 software (SPSS 1998). The entire image was selected for analysis by including all possible hue and saturation levels in the colour threshold option of the software. The average red, green, and blue measurement settings were used to obtain the average RGB levels for an image. The programmed formulas in the spread sheet converted absolute RGB levels (measured on a scale of 0 to 255) to percentage RGB levels by dividing each level by 255. Percentage RGB levels were then converted to average HSB levels by the following algorithms (Adobe Systems 2002):

Hue

Ifmax(R,G,B)=R,60{(G—B)/[max(R,G,B)—min(R,G,B)]}

Ifmax(R,G,B)=G,60(2+{(B—R)/[max(R,G,B)—min(R,G,B)]})

Ifmax(R,G,B)=B,60(4+{(R—G)/[max(R,G,B)—min(R,G,B)]})

Saturation

[max(R,G,B)—min(R,G,B)]/max(R,G,B)

Brightness

Max(R,G,B).

The plots were photographed on November 2013 in between 13.25 and 13.35 h during overcast conditions (illuminance≈ 5000 lux). Calibrations of images were under taken in dark conditions using only the camera flash as a light source. Digital images were taken of each of the five mutants (DFR-C-440, DFR-C-444, DFR-C-445, DFR-C 446 and DFR-C-448). The images were analyzed for HSB levels using the methods described by Karcher *et al*. (2003). One-way ANOVA was performed using PROC GLM in SAS statistical software on the HSB and DGCI data sets, with mutated lines as the treatment variable. For a given colour parameter, differences were determined significantly among cultivars 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 mutants' differences (Freundand Wilson 1993).

RESULTS AND DISCUSSION

Visual rating based on NTEP

Distinguished differences were observed in each treatment in terms of colour, texture and growth habit. Based on the turf quality, i.e. good density, fine leaf texture, slow growth and pendent growing habits, all selected mutants (DFR-C-440, DFR-C-443, DFR-C 444 DFR –C-446 and DFR-C-448) were found superior to parent (Fig 1). Among these DFR-C-444 was rated as superior followed by DFR-C-446. However, mutant DFR-C-448 had the slowest growth habit. A rating of 5 or above is considered minimally acceptable (Morris 2002). 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 (Mirik *et al*. 2006). This visual rating scale is mainly a function of colour, 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).

Digital image analysis of colour

A dark green colour index (DGCI) was created from the HSB values to obtain a single value from digital image colour analysis for comparison with values from subjective visual ratings. The index was created to measure the relative dark green colour of an image using the following equation:

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

The colour index was calculated from the average of transformed HSB parameters. Each transformed parameter measures dark green colour on a scale of zero to one. Since, the hue of most turf grass images ranges between 60 (yellow) and 120 (green), the maximum dark green hue was assigned as 120. Therefore, the dark green hue transform was calculated as (hue- 60)/60, so that hues of 60 and 120 would yield dark green hue transforms of zero and one, respectively.

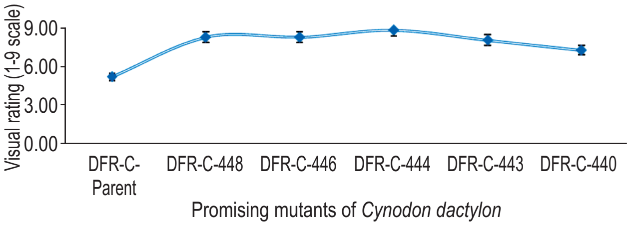

Fig 1 Average of turf quality in term of density, leaf texture, and colour texture of turf (*Cynodon dactylon*) based on NETP system of visual rating

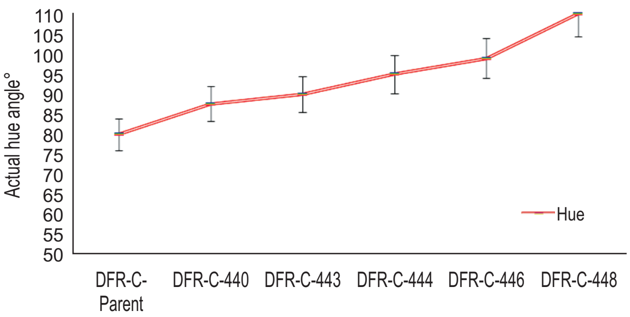

Fig 2 Hue angle quantified by digital image analysis

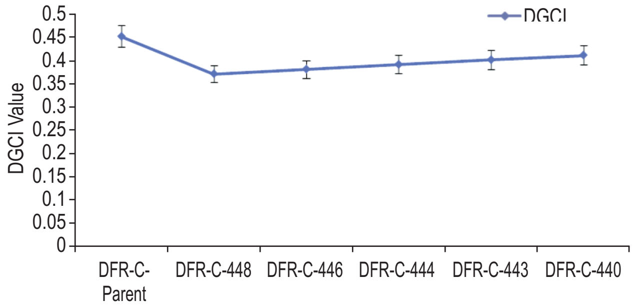

Fig 3 Dark green colour index(DGCI) of selected mutatnts

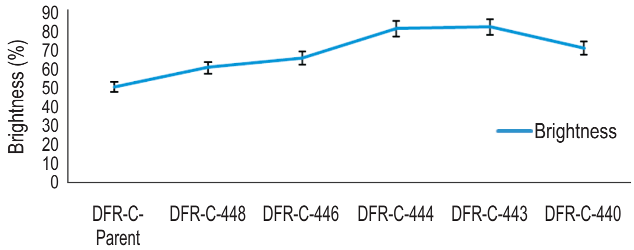

Fig 4 Colour brightness quantified by digital image

Since, lower saturation and brightness values corresponded to darker green colours (1-saturation) and (1-brightness) were used to calculate the dark green saturation and brightness transforms, respectively. The average of the transformed HSB values yielded a single measure of dark green colour, the DGCI value, which ranged from zero to one with higher values corresponding to darker green colour.

There were significant differences among mutants with regard to hue (Fig 2), saturation (Fig 3), brightness (Fig 4) and DGCI. The mutants differed significantly in HSB levels as compared to parent however, these differed significantly among themselves, only with regard to hue. Mutant's hue ranged from 80 to 110, while saturation and DGCI levels ranged between 47.44 to 49.99% and 0.37 to 0.43, respectively. Parent of mutants 'DFR-C-parent', with an average hue of 80^0 was significantly lighter in hue than all the five mutants. Among HSB, hue has been found to be the best indicator of the visual colour of a turf (Stafford *et al.* 2013). Amongst mutants, DFR-C-440 had a lighter shade of green to the eye than others. DFR-C-448, which appeared darker to the eye than the other mutants, had a significantly lower saturation level than the parent (Fig 4). The dark colour of this mutant was apparently due to its greyish green colour (less saturation), rather than it being a darker shade of green (higher hue). The DFR-C-448, DGCI mean ranked significantly lower than the parent; however it was non-significant with other mutants. These differences in colour are in strong agreement with results of visual rating where all mutants were significantly different than parent. This may be due to mutation amongst the selections. In mutants significant DGCI differences existed. The significant and positive correlation of visual ratings and hue of all mutants analyzed by digital image were obtained whereas it was negative and significant for brightness, saturation and dark green colour index (Table 1).

The significantly greater CV (%) with visual ratings suggests that rating values were evaluator dependent and that evaluators are likely to vary in ranking different shades of green (Goodenough and Goodenough 2012). Colour evaluation with digital photography and image analysis may minimize variations due to locations and years and would increase the validity of comparing colour data across both. The ability to distinguish colour differences among turf variants as H, S or B differences is a significant advantage of digital image analysis over subjective visual ratings. A turf that has a darker colour because of grayish

Table 1 Correlation amongst various visual rating (Quality, colour, texture) and digital image analyzed parameter

	Quality	Colour	Texture	Hue	Saturation value	Brightness	DGCI
Quality	1						
Colour	0.990728	1					
Texture	0.976306	0.986551	1				
Hue	0.731101	0.736044	0.790452	1			
Saturation value	–0.93007	–0.94733	–0.94016	–0.53985	1		
Brightness	0.716957	0.646679	0.644285	0.789732	–0.42006	1	
DGCI	–0.92004	–0.94674	–0.95647	–0.60822	0.990928	–0.4144	1

Table 2 Comparison of variance between subjective raters and digital image analysis for colour evaluation of *Cynodon dactylon* mutants

Particulars	Visual rating			Hue	Saturation value	Brightness	DGCI
	Quality	Colour	Texture				
Sampling information							
Sub sampling units	12	12	12	12	8	8	8
Experimental units	6	6	6	6	6	6	6
n	72	72	72	72	72	72	72
df	66	66	66	66	66	66	66
Statistics							
Mean(x-)	7.783	7.87	7.97	88.44	46.96	67.66	0.4
Confidence interval (95%) for sample mean	8.87-7.33	8.99-7.66	8.77-7.89	47.99-45.11	95.5-87.66	81-60	0.41-0.37
S	1.346	1.421	1.351	7.5	0.469	0.69	0.0196
CV %	23.11	26.33	25.66	1.8	4.4	1.79	0.008

genetic colour may not be desirable as that of is lighter in appearance but is saturated with green colour. Consequently, there exists a potential for evaluator bias, which may have occured due to varying evaluator perceptions of optimal dark green colour of the grass.

When visual ratings and digital image analysis were both performed, the statistical ranking of treatment means were similar between the two methods. However, DGCI variance was significantly lower than rater variance. Therefore, the DGCI is a more consistent measure of dark green colour across species than the individual measurements of H, S or B. Colour measurement using digital image analysis may be capable of assessing the N status of plant tissues (Karcher and Richardson 2003). In most cases, raters ranked the turf plants similarly although differences existed in their absolute rating values. Therefore, colour ratings remain a valid evaluation tool if data are not compared across raters. However, the accuracy of digital image analysis, demonstrated in the calibration experiments, enables researchers to record reflected turf grass colour on a standardized scale rather than using arbitrary rating values. Therefore, valid comparisons of colour data across researchers, locations, and years are possible with digital image analysis.

CONCLUSION

A digital image of various mutants, varied in visual colour due to genetically controlled differences which was quantified by digital image analysis and visual rating. Based on these analysis it may be concluded that the dwarf mutant had better quality lower canopy height, shorter internodes and shorter leaves than the parent. It is demonstrated that image analysis is a suitable tool to assess turf grass colour in a reproducible and calibrated manner, over a wide span of structural and colour attributes of turf grass.

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