HortFlora Research Spectrum, 3(3): 211-217 (September 2014)
www.hortflorajournal.com
ISSN : 2250-2823

COMPARING DIGITAL IMAGE ANALYSIS AND VISUAL RATING OF GAMMA RAY INDUCED PERENNIAL RYE GRASS (*Lolium perenne*) MUTANTS

Ajai Kumar Tiwari*, Gunjeet Kumar, Ganesh B. Kadam and Tarak Nath Saha

Directorate of Floricultural Research, College of Agriculture Campus Shivaji Nagar Pune -410 005
**E-mail: drajaitiwari@gmail.com*

ABSTRACT : To generate variability in perennial rye grass and to select genotypes responsive to low management, gamma-ray irradiation was used for induction of dwarfness and other quality attributes. The main objective of this study was to identify changes and correlations among turf visual rating and digital image analysis in evaluating turf grass quality under different treatments. Differences were significant among irradiated population with respect to hue angle, brightness and saturation. The correlations of hue and DGCI were significantly positive with all 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 tune 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 hue (r^2 = 0.3531) DGCI and saturation (r^2 = 0.3017); DGCI and brightness (r^2 = 0.1196) and DGCI and colour (r^2 = 0.1725). Non-linear relationship was noticed between DGCI and quality (r^2 = 0.0004).

Keywords : *Lolium perenne, turf quality, digital image analysis, dark green colour index.*

Traditional methods of determining turf quality have often been based on a visual rating system as per the National Turf grass Evaluation Program (NTEP) with a scale ranging from 1 to 9, with 1 representing the lowest quality and 9 representing the highest quality turf. A rating minimum of 5 is minimally acceptable (Morris, 10).This scale is mainly a function of colour, density, and uniformity (Horst *et al.,* 5). Differences in assessments by humans occur because of differences in individuals capability to perceive wave lengths of visible light, which lead to differences in visual estimates (Mirik *et al.,*9).Therefore, this rating system is biased due to subjectivities of the rater (Keskin *et. al.,*8).However, visual assessments are fast and easy to perform (Stafford and Goodenough, 4).

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.,* 1).

Digital image analysis (DIA) provides an alternative method to measure the reflectance from vegetated surfaces. Karcher and Richardson (7) found that DIA showed strong agreement with visual ratings in evaluating turf colour. An index known as the dark green color index (DGCI) was developed by Karcher and Richardson (7) by using hue, saturation, and brightness (HSB) levels. 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.,* 2). 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. Steddom *et al.* (13) 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 or RAW images. Therefore, digital photography and subsequent image analysis maybe 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 Lolium perenne population by use of digital camera image analysis and supported by the software using an HSB colour scale.

MATERIALS AND METHODS

Physical Mutagen Treatment

Irradiation with gamma rays of *Lolium perenne* with Co^{60} was done on 30 uniform stolen (sprigs) sets of propagules for each treatment. These were

Received : 10-08-2014 *Accepted : 04-09-2014*

irradiated with nine doses (5.0, 7.5, 10.0, 12.5, 15.0, 17.5, 20.0, 22.50, 25.00 KRD) of gamma rays at National physical laboratories, Indian Agricultural Research Institute, New Delhi during October 2012, subsequently the mutants were selected and named as T_1 to T_9, respectively. 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 × 2 meter beds with three replications of each treatment.

Visual Rating for Quality

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 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 evaluated.

Digital Image Analysis

Turf quality was evaluated by using digital image analysis process that inclyde; (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) developed by Karcher and Richardson (7).

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

Where:

DGCI=dark green color index,

H,S,B=hue, saturation, and brightness levels

All digital images in these studies were taken with a CANONEOS 60D camera. The images were collected in JPEG format, with a color depth of 16.7million colors, 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 off /2.8, and a focal length of 80mm. All images were captured using a uniform light source (Ikemura, 6) 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.

Camera Calibration and Treatments Colour Differences

The plots were photographed on November 2013 in between 13.25 and13.35 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 (7). 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, 3).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 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 and mean are depicted in Fig. 1. All five evaluators observed differences in color and texture of the mutants. Based on the turf color and texture mutant t_9 followed by mutant t8 were superior over others (Fig. 1). The data indicated that the variances were higher for all the visual rating parameters *i.e.* quality, color and texture. The lowest values of variances were recorded in DGCI and hue (Table 1). There were significant differences among mutants with regard to HSB and DGCI. Amongst mutants, t_5 had maximum hue followed by the mutant t_6 (Fig. 1).

The Pearson correlation statistics along with Fisher's Z transformation amongst DIA (HSB, DGCI) and NETP visual ratings 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 correlation of brightness with quality and texture, and 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,

Table 1: Descriptive statistics of various parameters as affected by as affected by various treatments

	Treatment	t_0	t_1	t_2	t_3	t_4	t_5	t_6	t_7	t_8	t_9
Mean	Quality	7.000	7.000	7.400	7.400	7.000	6.600	6.200	6.200	7.000	7.600
	Colour	6.800	6.200	7.400	7.000	7.800	7.200	7.000	7.000	7.800	8.600
	Texture	6.600	6.800	7.800	7.600	7.000	5.600	5.800	7.000	7.000	7.000
	Hue	53.942	58.196	57.454	57.270	60.800	78.446	61.016	63.893	51.42	23.912
	Saturation	37.253	31.990	27.463	32.321	29.588	25.929	18.281	36.229	19.898	13.514
	Brightness	186.400	180.600	197.400	190.40	195.80	175.00	192.2	175.200	196.0	210.200
	DGCI	0.451	0.453	0.455	0.453	0.452	0.451	0.453	0.453	0.463	0.463
Standard Deviation	Quality	1.000	0.707	0.548	0.548	0.707	1.673	0.837	1.304	1.414	1.140
	Colour	1.304	0.837	0.894	0.707	0.447	0.447	0.000	0.000	0.447	0.548
	Texture	0.548	0.837	0.447	0.548	0.707	0.548	0.837	1.000	0.707	1.000
	Hue	0.527	0.280	0.789	0.942	0.426	0.527	0.547	0.274	0.531	9.083
	Saturation	0.760	0.661	0.836	0.566	0.625	0.428	0.737	1.089	1.171	1.196
	Brightness	8.989	2.881	15.110	21.256	15.023	9.274	18.295	2.280	14.053	3.564
	DGCI	0.003	0.003	0.001	0.004	0.004	0.006	0.005	0.005	0.004	0.006
Standard Error	Quality	0.447	0.316	0.245	0.245	0.316	0.748	0.374	0.583	0.632	0.510
	Colour	0.583	0.374	0.400	0.316	0.200	0.200	0.000	0.000	0.200	0.245
	Texture	0.245	0.374	0.200	0.245	0.316	0.245	0.374	0.447	0.316	0.447
	Hue	0.235	0.125	0.353	0.421	0.190	0.236	0.245	0.123	0.237	4.062
	Saturation	0.340	0.296	0.374	0.253	0.279	0.191	0.330	0.487	0.524	0.535
	Brightness	4.020	1.288	6.757	9.506	6.719	4.147	8.182	1.020	6.285	1.594
	DGCI	0.001	0.002	0.000	0.002	0.002	0.002	0.002	0.002	0.002	0.003
Variance	Quality	1.000	0.500	0.300	0.300	0.500	2.800	0.700	1.700	2.000	1.300
	Colour	1.700	0.700	0.800	0.500	0.200	0.200	0.000	1.000	0.200	0.300
	Texture	0.300	0.700	0.200	0.300	0.500	0.300	0.700	1.000	0.500	1.000
	Hue	0.277	0.078	0.622	0.888	0.181	0.278	0.299	0.075	0.282	82.504
	Saturation	0.577	0.437	0.700	0.321	0.390	0.183	0.543	1.187	1.372	1.430
	Brightness	80.800	8.300	228.300	451.80	225.70	86.000	334.700	5.200	197.5	12.700
	DGCI	0.000008	0.0000117	0.0000012	0.000013	0.000014	0.0000305	0.000021	0.000022	0.000019	0.000032

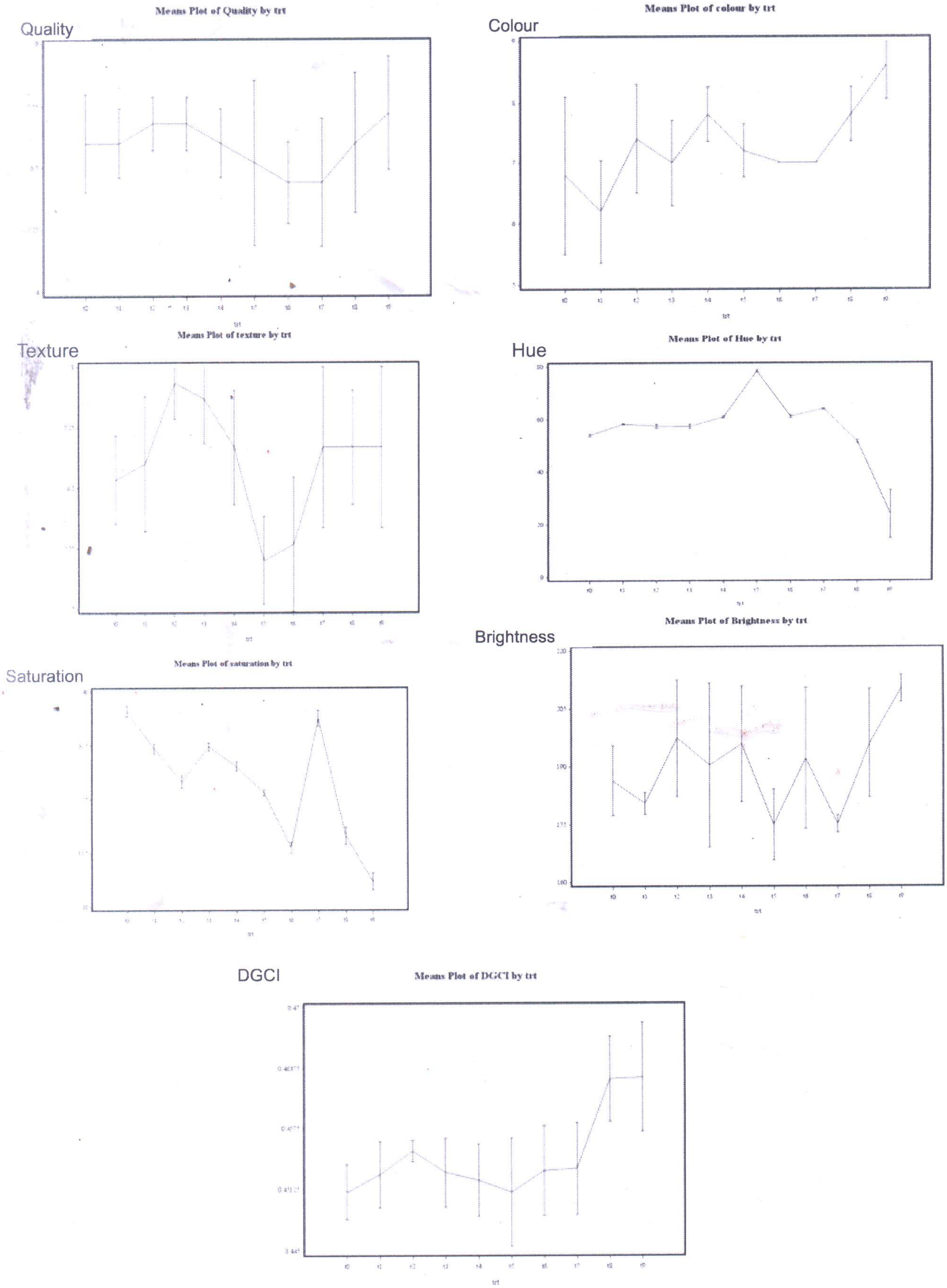

Fig. 1 : Mean plot of various parameters as affected by treatments.

(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, 3). These relationships were better in DGCI and hue (r^2= 0.3531) DGCI and saturation(r^2= 0.3017); DGCI and brightness (r^2 = 0.1196) and DGCI and colour (r^2 = 0.1725). Non-linear relationship was noticed between DGCI and quality (r^2 = 0.0004).

The significantly greater variances 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, 4). 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.*, 9).This visual rating scale is mainly a function of color, density, and uniformity (Newton, 11). This rating system is biased due to subjectivities of the raters and has inaccurate estimation of turf quality (Keskin *et al.*, 8).Among HSB, hue has been found to be the best indicator of the visual color of the turf (Stafford *et al.*, 12). 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.

Table 2: Pearson Correlation Statistics (Fisher's z Transformation)

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	0.12782	0.12852	0.0013	0.12654	-0.157354	0.391108	0	0.3783
Quality	Texture	50	0.02994	0.02995	0.0003055	0.02963	-0.250782	0.305462	0	0.8373
Quality	Hue	50	-0.31126	-0.32194	-0.00318	-0.30839	-0.540351	-0.032859	0	0.0273
Quality	Saturation	50	-0.09369	-0.09396	-0.000956	-0.09274	-0.36175	0.190525	0	0.5195
Quality	Brightness	50	0.18265	0.18473	0.00186	0.18085	-0.102665	0.437191	0	0.2054
Quality	DGCI	50	0.02113	0.02113	0.0002156	0.02092	-0.258939	0.297532	0	0.8848
Colour	Texture	50	-0.01181	-0.01181	-0.0001205	-0.01169	-0.289097	0.267529	0	0.9355
Colour	Hue	50	-0.43953	-0.47165	-0.00449	-0.43591	-0.63697	-0.179318	0	0.0012
Colour	Saturation	50	-0.48926	-0.53509	-0.00499	-0.48545	-0.672879	-0.239464	0	0.0002
Colour	Brightness	50	0.51187	0.56526	0.00522	0.50801	0.267482	0.688938	0	0.0001
Colour	DGCI	50	0.41539	0.4421	0.00424	0.41187	0.150815	0.619229	0	0.0024
Texture	Hue	50	-0.2792	-0.28681	-0.00285	-0.27657	-0.51525	0.001929	0	0.0493
Texture	Saturation	50	0.1631	0.16457	0.00166	0.16148	-0.122371	0.420906	0	0.2592
Texture	Brightness	50	0.13935	0.14026	0.00142	0.13796	-0.145998	0.400909	0	0.3363
Texture	DGCI	50	0.22938	0.23353	0.00234	0.22716	-0.054645	0.475444	0	0.1094
Hue	Saturation	50	0.48411	0.52834	0.00494	0.48032	0.233141	0.669198	0	0.0003
Hue	Brightness	50	-0.52741	-0.58655	-0.00538	-0.52351	-0.699874	-0.286981	0	<.0001
Hue	DGCI	50	0.5942	0.68413	0.00606	0.59026	0.746038	0.373237	0	<.0001
Saturation	Brightness	50	-0.43489	-0.46591	-0.00444	-0.43129	-0.633575	-0.173804	0	0.0014
Saturation	DGCI	50	-0.54926	-0.61732	-0.0056	-0.54533	-0.71513	-0.314765	0	<.0001
Brightness	DGCI	50	0.34588	0.36076	0.00353	0.34277	0.071221	0.567021	0	0.0134

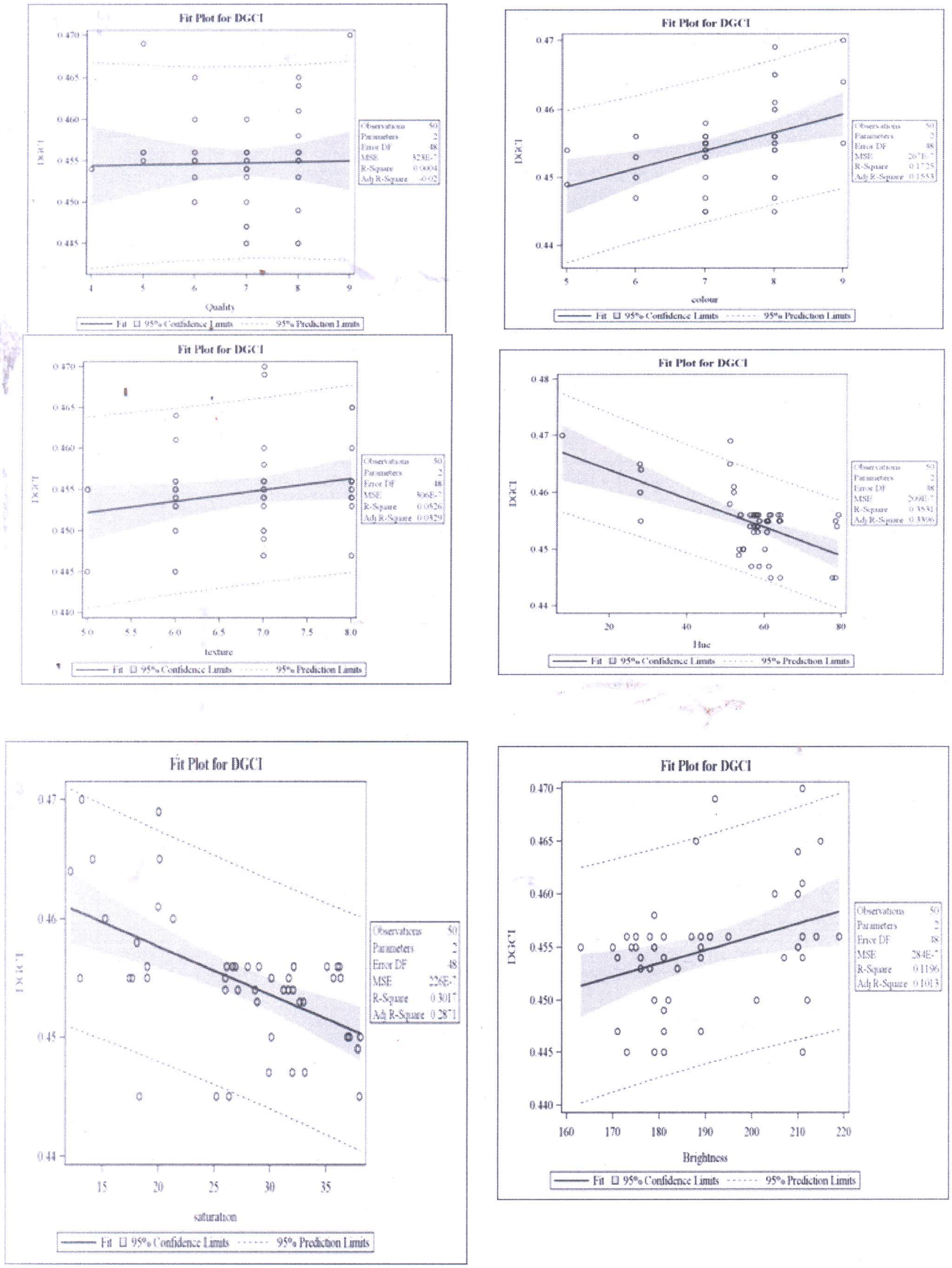

Fig. 2 : Linear Regression of various parameters using DGCI as dependable variable.

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, non-destructive, 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.,* 2). 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 (7).The evaluation and comparison of the both turf quality evaluation techniques that were considered in this study enabled us to draw the following conclusions about the digital imagery process.

- ✓ Digital image analysis provides objective, quantitative turf quality evaluation and little to no prior experience is needed. On the other hand, visual rating technique needs substantial training and measurements 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 reported on a discrete scale, but DGCI of DIA were reported turf quality on a continuous scale which brings turf quality estimates to more realistic measurements.
- ✓ 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 turf grass colour.

REFERENCES

1. DaCosta, M., Wang, Z., and Huang. B., (2004). Physiological adaptation of Kentucky bluegrass to localized soil drying. *Crop Sci.,* **44** : 1307–1314.
2. Díaz - Lago, J.E., Stuthman, D.D., Leonard, K.J., (2003). Evaluation of components of partial resistance too at crown rust using digital image analysis. *Plant Dis.,* **87** : 667–674.
3. Freund, R.J. and Wilson, W.J. (1993). *Statistical Methods.*Academic Press, San Diego, CA.
4. Goodenough, A. E. and Goodenough, A. S. (2012). Development of a rapid and precise method of digital image analysis to quantify canopy density and structural complexity. ISRN Ecology Article ID 619842:11 http://dx.doi. org/ 10.5402/2012/619842.
5. Horst, G.L., Engelke, M.C. and Meyers, W. (1984). Assessment of visual evaluation techniques. *Agron.J.,* **76** : 619-621.
6. Ikemura, Y. (2003). Using digital image analysis to measure the nitrogen concentration of turf grasses. *M.S. thesis.* Univ.of Arkansas, Fayetteville.
7. Karcher, D.E. and Richardson, M.D. (2003). Quantifying turf grass colour using digital image analysis. *Crop Sci.,* **43** : 943–951
8. Keskin, M., Dodd, R.B., Han, Y.J. and Khalilian (2003). Predicting visual quality ratings of turfgrass research plots using spectral reflectance. Paper Number: 031114, ASABE Meeting Presentation.
9. Mirik, M, Michels, GJ.J, KassymzhanovaMirik, S., Elliott, N.C., Catana, V., Jones, D.B. and Bowling, R, (2006). Using digital image analysis and spectral reflectance data to quantify damage by greenbug (Hemitera: Aphididae) in winter wheat. *Computers and Electronicsin Agri.,* **51** : 86-98.
10. Morris, K.N. (2002). *A guide to NTEP Turf grass Ratings.* The National Turfgrass Evaluation Program (NTEP).
11. Newton, A. C. (2007). *Forest Ecology and Conservation: A Handbook of Techniques,* Oxford University Press, Oxford, UK,
12. Stafford, R., Adam, G. H., and Goodenough, AE (2013). Using Long-Term Volunteer Records to Examine Dormouse (*Muscardinus avellanarius*) Nestbox Selection, *Public Library of Sci.* **8**(6)
13. Steddom, K., McMullen, M.,Schatz, B. and Rush, C.M., (2004). Assessing foliar disease of wheat image analysis. In: The 2004 Summer Crops Field Day at Bush land, TX Sponsored by the Cooperative Research, Education & Extension Team (CREET), Bush land, TX, pp.32 – 38.

Citation : Tiwari A.K., Kumar G., Kadam G.B. and Saha T.N. (2014). Comparing digital image analysis and visual rating of gamma ray induced perennial rye grass (*Lolium perenne*) mutants. *HortFlora Res. Spectrum,* **3**(3) : 211-217.