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Flower colour variations in gerbera (*Gerbera jamesonii*) population using image analysis

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ABSTRACT

Image analysis was performed on ligules of 211 progenies and seven parental accessions of gerbera (*Gerbera jamesonii* Bolus ex. Hooker F), to quantify colour variation. The colour variations were assessed based on mean values of (1) red, green, blue (RGB), (2) hue and saturation (HS) colour channels of ligule colour. Multivariate clustering of genotypes based on RGB and HS colour variables determined the relative position of genotypes in the paired group cluster. The genotypes grouped into different clusters representing distinct colour sub-groups which could be distinguished from each other based on Student's 't' test. Two major clusters were observed which correspond to the flavonoid and carotenoid pigments which contribute to flower colour variations in gerbera. Analysis of variance among genotypes within each colour cluster revealed significant variations for ligule colour implying high degree of polymorphism for the trait but the extent of variation was lower compared to variations among clusters. Ten pairs of genotypes were identified with similar colour expression, which have potential use in breeding for homogeneous colour expression of gerbera populations.

Key words: Flower colour variations, Gerbera, Image analysis, RGB and HSV quantification, RHS colour chart

Flower colour is an important breeding objective in gerbera (*Gerbera jamesonii* Bolus ex. Hooker F) and many varieties have been bred to obtain desirable colour types. Random mating populations of gerbera show a pattern of continuous variation for flower colour necessitating resort to biometrical genetic analysis to elucidate modes of inheritance. Flower colours are generally determined in the framework of DUS (distinctiveness, uniformity and stability) testing by comparing petal colour with the Royal Horticultural Society (RHS) colour chart (1995). Such colour evaluations are categorical and subject to drawbacks such as non-availability of all colours, difficulty in describing subtle variations, lack of statistical analysis of data and errors due to variations in light intensity that affect the results (Lootens *et al*. 2007). These drawbacks may effectively be overcome by adopting image analysis which is simple, objective and quantitative evaluation technique and serves as an efficient tool to quantify colour variations. Image analysis is being used world wide for divergent agricultural applications such as colour and/or shape pattern analysis in *Primula* (Yoshioka *et al*. 2004), lisianthus (Yoshioka *et al*. 2006), gerbera (Lino *et al*. 2011), fruits (Blasco *et al*. 2007, 2009) and variety recognition of roses (Zhenjiang *et al*. 2006). More recently, use of automated computer system to introduce machine vision for flower classification tasks is being researched (Guru *et al*. 2010, Gracia *et al*. 2011).

In the present study, an attempt was made to highlight the range of flower colour variations in gerbera population using image analysis by defining the colour classes and also evaluate genotypic variability in different colour groups by quantifying flower colour.

MATERIALS AND METHODS

To assess flower colour variations in gerbera, plant material was generated from crosses in different combinations among seven gerbera accessions (IHBT-Gr 1 to IHBT-Gr 7) which were identified on the basis of flower colour and shape from a collection of gerbera genotypes procured from New Delhi and being maintained at the Institute of Himalayan Bioresource Technology, Palampur. The colour descriptions of parents evaluated on the basis of RHS colour chart plus RGB and HS quantification are presented in Table 1. A total of 211 genotypes representing 23 different cross combinations along with parental accessions were taken for the study to

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Table 1 RHS colour descriptions and mean values of RGB and HS colour variables of parental accessions

Parent	RHS description	Colour group	Red	Green	Blue	Hue	Saturation
IHBTGr1	155C	White	252.76	251.42	231.52	41.00	21.00
IHBTGr2	58D	Red purple	254.11	136.44	174.24	241.00	118.00
IHBTGr3	23A	Yellow orange	246.79	167.92	44.51	26.00	210.00
IHBTGr4	33A	Orange red	238.46	78.41	73.03	1.00	177.00
IHBTGr5	66B	Red purple	214.10	58.53	138.35	233.00	186.00
IHBTGr6	58D	Red purple	248.50	145.62	173.44	244.00	106.00
IHBTGr7	52C	Red	251.38	139.86	159.82	247.00	113.00

assess variations for ligule colour. Observations were recorded on ligules of three flower replicates per genotype over three growing seasons (October 2008, March 2009 and October 2009) at the flowering stage when colour of ray florets is fully developed.

The ligules were detached from their base and images were obtained in digital image scanner by placing them on surface of the scanner and scanning at 600 dpi resolution (as per the procedure suggested by Yoshioka *et al*. 2004). The images were saved as true colour images in TIFF format to include all the variations available in the ligule colour and further analyzed in Photoshop 7.0 programme.

Since the ligules are of different shape and size, each scan has a different pixel size. Further, the colour distribution is not uniform throughout the ligule surface. In order to homogenize the data set, the scans were divided into grids of 9801 pixels size and selection area of 9801 pixels was demarcated per ligule in the region where colour appeared to be most uniform. The colour observations were made from these selected area demarcations of ligules by constructing a histogram for each colour channel from the selected area and means of the red, green and blue (RGB) colour channels were recorded (Lootens *et al*. 2007). Using RGB colour calculator data of hue, saturation and value (HSV) colour channels were also worked out. The observations of red colour channel (R) correspond with those of value (V) therefore, observations of only red colour channel were taken into consideration for further analysis.

In order to confirm the stability of the light conditions for each scan, the repeatability of RGB colour variables were determined as the correlations between measurements made over two replicates on parental genotypes. Student's t test was also done to compare the measurements of RGB variables made over the two replicates and assess variations in light intensity if any. Alternatively, control colour patches were also scanned at regular intervals of ligule scans to test the stability of the scanning light as per Yoshioka *et al*. (2004).

To compare flower colour, ligule colours of all the 211 genotypes along with seven parental accessions were quantified into RGB and HS colour variables and the mean values of variables contributing to colour expression of genotypes were used for multivariate clustering based on paired group cluster method using similarity co-efficient under Past 1.40 software (Hammer *et al*. 2001). Differences among clusters was determined by using Student's 't' test (based on common standard deviations for comparison of mean values of adjacent clusters with varying number of genotypes). Variations among genotypes within the clusters were determined by F test and analysis of variance was performed using CPCS programme. Similarity of colour among genotypes of a colour group was evaluated on the basis of critical difference values of the respective colour groups.

RESULTS AND DISCUSSION

A wide range of flower colour variations was generated through hybridization programme in gerberas using the seven parental accessions. Regarding the light stability test, correlations between measurements of RGB variables made over two replicates of parental genotypes were 0.98, 0.99 and 0.99, respectively. Based on Student's t test (t value of RGB variables – 0.015, 0.236 and 0.004, respectively) no significant differences were found between the replicates suggesting that the replicates were at par with each other and the light condition was stable during experimentation. Also, using scans of control colour patches stable light intensity was observed for all the scans. The results conform to earlier studies on stability of light intensity (Yoshioka *et al*. 2004).

Based on visual observations of the population, gerbera ligule colour could be broadly distinguished into five major colour groups, viz white, yellow, orange, red, and red purple groups. However, quantification of ligule colours into RGB and HS colour variables and multivariate clustering of 211 genotypes along with parental accessions distinguished the genotypes into different colour clusters (Fig 1) which correspond to different colour groups as per descriptions of the RHS colour chart. The results imply that parameters based on the RGB and HS colour variables present the largest discriminating power as suggested by Lootens *et al*. (2007).

No parental accessions were present in clusters of red and yellow colour groups, while two of the parental accessions IHBT Gr1 and IHBT Gr3 did not pair with any of the progeny genotypes implying that a significant range of flower

colour variations can be achieved in gerberas through a hybridization programme. White, yellow and yellow orange colour groups formed fragmented clusters suggesting possibility of allelic variations or interactions among the flower colour pigments in the genotypes under these groups. The overall expression of flower colour variations suggests polygenic inheritance of the trait. Earlier reports in gerbera suggest that distributions of the colour attributes in the population are bimodal (Tourjee *et al*. 1995), consistent with the presence of a gene with major effect segregating in a background of polygenic variation.

Since colour differences between white, yellow, orange, red, and red purple are large, clusters of these groups can be distinguished on visual basis. However, variations in colour of sub-clusters with narrow differences (white – orange white, yellow 2 – yellow 3, orange – orange red, yellow orange 4 – red purple 1, red purple 1 – red purple 2, red purple 4 – red purple 5, red purple 5 – red2, red 1 – red 3 and red 2 – red 3) need to be evaluated statistically to determine variations among the clusters. Therefore, Student's 't' test was applied which distinguished these sub-clusters revealing significant variations among them (Table 2). Image analysis can thus evaluate all colours present without being biased by an expert (Lootens *et al*. 2007).

Two major clusters were observed (Fig 1), first cluster with genotypes having ligule colour in white, yellow, yellow orange, orange and orange red colour groups while second cluster comprised genotypes having ligule colour in red and red purple colour groups. Based on mean values of different clusters (Fig 2), H distinguished all the genotypes into two major groups. In one group, mean values of H range from 4.00 to 36.11 which includes clusters of white, yellow, orange white, yellow orange, orange and orange red colour groups. In the second group range of H is beyond zero (235.72 to 251.00, considering channel circularity) for clusters of red and red purple colour groups. Other than hue, the distinguishing feature among these two major clusters was that mean values of green colour channel were higher than those of blue in the former, whereas the situation was reverse in the latter group.

Formation of two major clusters suggests the role of two major loci contributing to colour variations. Earlier studies demonstrated in gerbera that the red ligule colours are produced by flavonoid pigments and the yellows by carotenoids (Tyrach and Horn 1997) which are two distinct biochemical pathways implying bimodal inheritance of ligule colour. Tourjee *et al*. (1995) suggested that a putative major gene may be controlling the expression of flavonoid pigments which may mask the expression of carotenoid genes.

The distribution pattern of hue in the present study suggests that the masking effect is not complete and there is co-expression of both the flavonoid and carotenoid pathways in the genotypes under orange white, yellow orange, orange and orange red colour group clusters where the mean value

Table 2 Comparison of different colour cluster means in gerbera population on the basis of t test

Clusters	df	Red			Green			Blue			Hue			Saturation		
		X	CSD	t	X	CSD	t	X	CSD	t	X	CSD	t	X	CSD	t
White/orange white	31	3.56	4.01	2.52*	0.56	3.66	0.43	9.63	4.32	6.31*	1.36	6.92	0.55	13.84	3.09	12.70*
Yellow2/Yellow3	24	2.74	6.88	0.96	7.45	8.15	2.21*	32.31	9.09	8.62*	0.54	3.18	0.40	31.53	9.67	7.90*
Orange/orange red	25	2.34	7.70	0.61	22.90	21.60	2.13*	43.34	64.95	1.34	4.61	4.34	2.14*	45.29	13.55	6.74*
Yellow orange4/red purple1	15	2.66	5.12	0.97	30.38	10.77	5.29*	13.14	10.50	2.34*	236.93	4.78	93.05*	24.23	13.06	3.48*
Red purple1/red purple2	11	1.67	5.45	0.53	45.67	12.70	6.30*	29.14	9.62	5.31*	4.39	2.57	2.99*	48.52	13.12	6.48*
Red purple 4/ red 5 purple	31	2.12	7.34	0.64	3.18	12.49	0.56	36.77	10.19	7.99*	11.02	5.42	4.50*	2.28	12.60	0.40
Red purple5 / red2	26	0.73	5.96	0.26	30.29	8.02	8.19*	19.20	8.37	4.98*	2.10	2.20	2.07*	32.83	8.61	8.27*
Red1/red3	20	15.80	9.55	3.25*	38.18	7.72	9.70*	37.63	7.06	10.47*	4.89	1.79	5.37*	43.13	9.40	9.01*
Red2/red3	37	12.35	7.77	4.91*	22.42	8.26	8.39*	26.49	8.17	10.03*	1.40	2.23	1.93	21.30	9.21	7.16*

X, Difference of Mean; CSD, common standard deviation; t, t value (calculated); *$P = 0.05$

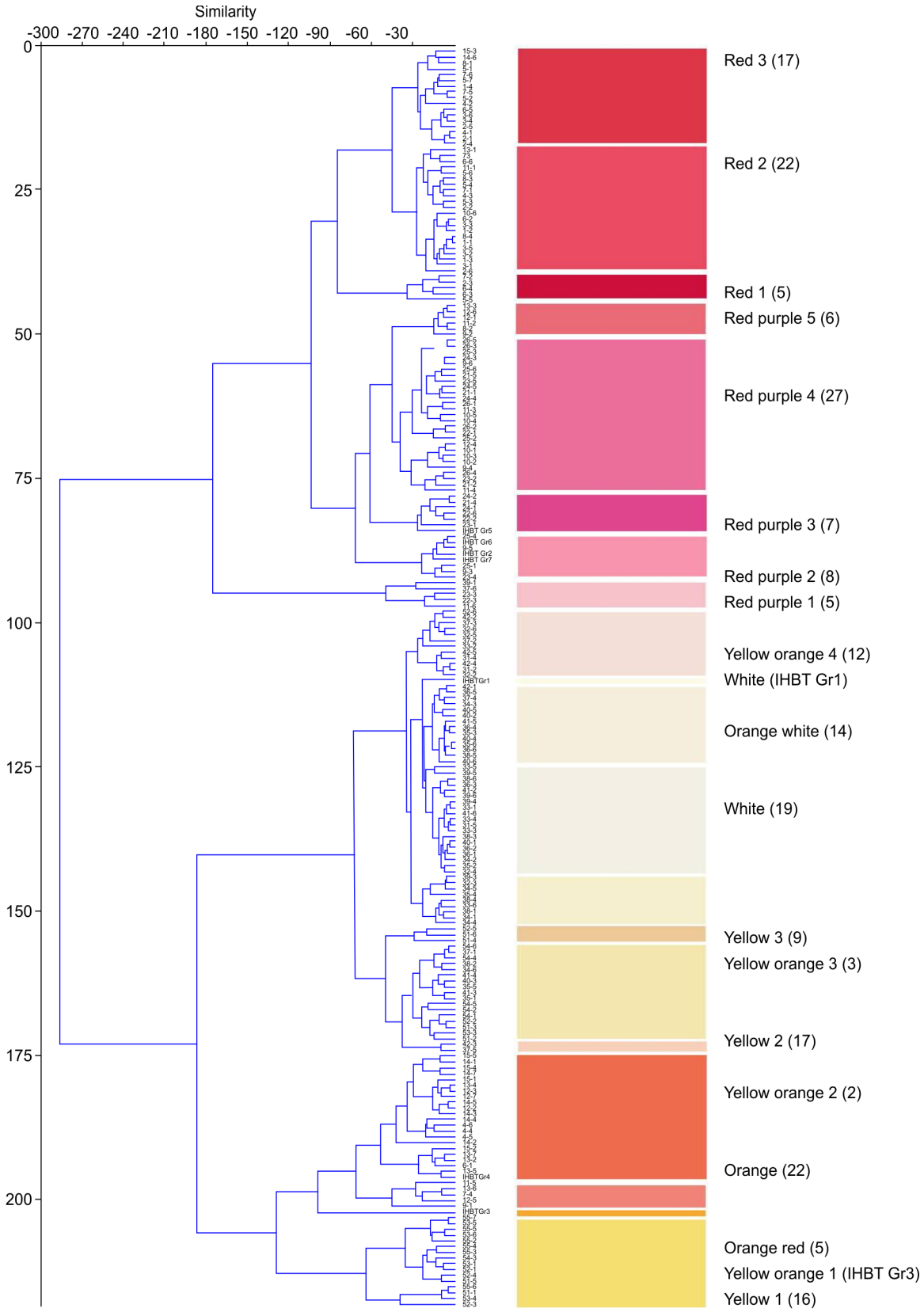

Fig 1 Multivariate clustering and distinction of different colour groups based on image analysis of ligule colour in gerbera (no. of genotypes in parentheses)

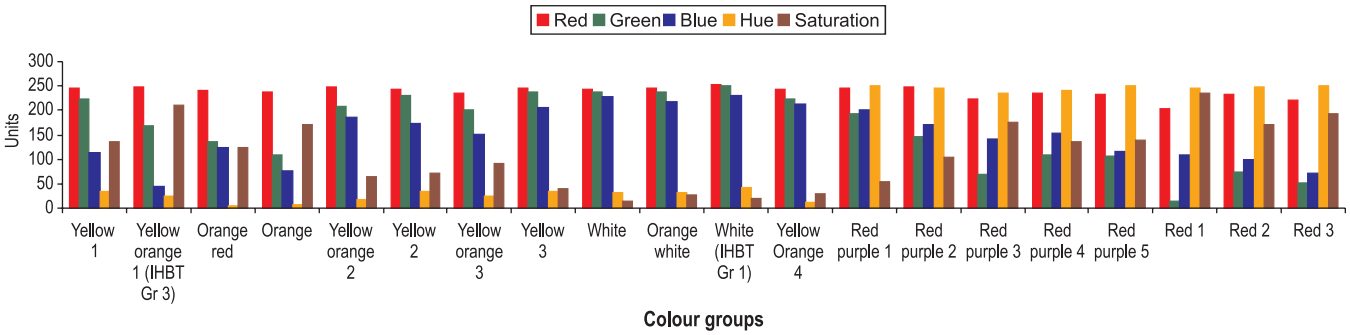

Fig 2 Mean values of RGB and HS colour channels for different colour group clusters

of hue approaches zero (considering channel circularity) in comparison to higher mean values of hue in groups with white and yellow colours.

Variations within the clusters were evaluated in 16 clusters, viz white, yellow (3 clusters), orange white, yellow orange, orange, orange red, red (3 clusters) and red purple (5 clusters) colour groups. Based on F value, significant variations were observed for RGB and HS variables within

Table 3 Analysis of variance of the means for genotypes in different colour groups

Source	df	Variance	F value	cd 5%	Mean	CV	df	Variance	F value	cd 5%	Mean	CV
White							*Yellow 1*					
R	18	50.78	31.27*	2.10	241.88	0.53	15	30.21	22.33*	1.93	244.57	0.48
G		39.60	21.45*	2.24	238.82	0.57		176.63	97.38*	2.24	223.36	0.60
B		64.54	23.33*	2.75	228.85	0.73		1623.47	114.45*	6.27	113.33	3.32
H		153.56	11.87*	5.95	32.38	11.11		24.08	71.37*	0.96	35.70	1.63
S		27.74	11.07*	2.62	13.87	11.41		1766.25	108.14*	6.73	136.77	2.95
Yellow 2							*Yellow 3*					
R	16	153.95	112.79*	1.94	242.59	0.48	8	117.94	99.18*	1.88	245.33	0.44
G		197.25	124.34*	2.09	231.44	0.54		203.18	168.88*	1.89	238.89	0.46
B		324.75	21.54*	6.45	174.08	2.23		93.40	6.65*	6.48	206.39	1.82
H		24.89	31.59*	1.47	35.64	2.49		41.41	17.75*	2.64	35.11	4.35
S		377.92	23.20*	6.71	71.94	5.61		86.48	5.71*	6.73	40.40	9.63
Orange white							*Yellow orange 4*					
R	13	44.31	29.18*	2.06	245.44	0.50	11	61.25	26.95*	2.55	243.84	0.62
G		40.83	36.11*	1.78	238.27	0.45		97.20	49.59*	2.37	223.44	0.63
B		47.98	13.51*	3.16	218.86	0.86		238.12	73.06*	3.05	214.25	0.84
H		130.33	56.57*	2.54	31.02	4.89		85.63	10.51*	4.83	12.33	23.15
S		29.99	6.31*	3.65	27.71	7.87		353.18	76.18*	3.64	30.83	6.98
Orange							*Orange red*					
R	21	178.78	70.61*	2.62	238.17	0.67	4	174.90	72.69*	2.92	240.52	0.64
G		1570.16	332.13*	3.58	107.88	2.02		506.92	159.89*	3.35	130.79	1.36
B		506.79	83.62*	4.05	76.34	3.22		503.42	215.38*	2.87	119.68	1.28
H		64.23	102.81*	1.30	8.34	9.47		16.56	21.61*	1.64	3.73	23.45
S		529.17	73.37*	4.42	173.36	1.55		664.56	438.32*	2.31	128.06	0.96
Red 1							*Red 2*					
R	4	284.52	30.57*	5.74	204.31	1.49	21	113.00	34.22*	2.99	232.46	0.78
G		233.86	27.73*	5.46	14.86	19.54		227.42	45.95*	3.66	75.45	2.95
B		228.25	9.26*	9.35	57.51	8.63		244.77	66.77*	3.15	98.44	1.94
H		2.07	2.71	NS	245.40	0.36		17.09	47.31*	0.99	248.89	0.24
S		366.39	25.98*	7.07	236.60	1.59		265.64	49.45*	3.81	172.16	1.35
Red 3							*Red purple 1*					
R	16	271.22	44.66*	4.09	220.11	1.12	4	126.30	35.90*	3.53	246.50	0.76

Continued

Table 3 *Concluded*

Source	df	Variance	F value	cd 5%	Mean	CV	df	Variance	F value	cd 5%	Mean	CV
G		176.42	20.84*	4.83	53.03	5.49		1038.07	361.27*	3.19	193.07	0.88
B		141.93	24.48*	4.00	71.96	3.35		587.41	125.41*	4.07	201.11	1.08
H		12.33	39.27*	0.93	250.29	0.22		21.90	16.85*	2.14	249.26	0.46
S		240.04	23.26*	5.34	193.47	1.66		948.56	201.11*	4.09	55.06	3.94
Red purple 2							*Red purple 3*					
R	7	68.30	41.21*	2.25	248.16	0.52	6	95.85	48.68*	2.49	222.90	0.63
G		167.21	83.30*	2.48	147.39	0.96		198.75	25.53*	4.96	69.22	4.03
B		101.18	63.85*	2.20	171.97	0.73		164.28	63.15*	2.87	140.69	1.15
H		18.66	41.80*	1.17	244.87	0.27		11.34	71.04*	0.71	235.33	0.17
S		269.68	91.15*	3.01	103.58	1.66		256.15	24.63*	5.73	175.95	1.83
Red purple 4							*Red purple 5*					
R	26	176.93	95.91*	2.22	235.32	0.38	5	80.82	41.63*	2.53	233.2	0.60
G		548.08	106.27*	3.72	108.92	2.08		49.49	6.7*	4.94	105.74	2.57
B		359.12	106.05*	3.01	154.42	1.19		65.57	15.55*	3.73	117.65	1.75
H		104.41	295.55*	0.974	239.97	0.25		4.26	6.74*	1.44	251.00	0.32
S		560.22	108.22*	3.72	137.04	1.66		41.46	5.34*	5.06	139.33	2.00

*$P = 0.05$

the clusters (Tables 3).

Regarding F test, variables with maximum variances in each colour group influenced the colour to a greater extent compared to other variables. However, observations based on variables with lower coefficient of variations tend to be more reliable as they are more stable for assessment of variation among genotypes. The F test confirms that quantification of colours using image analysis can detect even subtle colour differences which are not perceptible to the human eye. Similar results were obtained by Lootens *et al*. (2007) while evaluating flower colours of *Begonia* × *tuberhybrida* genotypes. Based on critical difference values, ten pairs of genotypes in different colour groups were identified which have colour expression at par with each other (Table 4). Hybridization among these pairs of genotypes will express the additive, dominance and interactive genic components of the respective colours contributing to inheritance of colour expression in gerbera. These pairs of gerbera genotypes have potential use in breeding for homogeneous colour of gerbera populations.

To conclude, colour evaluation using RHS colour chart is a common procedure for colour description worldwide which is a qualitative analysis. However, colour evaluations which exhibit continuous variations (as in gerbera) need a quantitative approach for evaluation so that variations can be grouped and then assessed for relative position within a particular group. The advantage of image analysis in the present study is that it provided increased precision for estimates of variation by differentiating certain colour groups, viz yellow, red and red purple into sub-groups which in turn allowed for determination of variations within the sub-groups in a reliable manner through F test. The procedure adopted in the present study provided an objective and simple approach for quantitative evaluation of flower colour, allowed assessment of genetic variability in the population and also helped in choice of parents for further breeding.

Table 4 Similarity among genotypes for ligule colour based on critical difference values

Colour group	Genotypes
White	39-4 and 33-1, 40-1 and 36-2
Yellow orange 4	42-4 and 31-2
Orange white	40-4 and 35-6
Orange	13-4 and 12-3
Red 3	4-1 and 2-1
Red purple 2	21-4 and 24-2
Red purple 4	26-3 and 26-5
Red purple 5	11-2 and 8-2, 12-6 and 13-3

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