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Assessment of genetic diversity using D^2 analysis in warm-season turfgrasses

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Abstract
The present experiment was conducted to estimate the variability and diversity in thirteen warm season turfgrass genotypes. The mean sum of squares due to genotypes showed significant differences for all the characters. High and moderate estimates of heritability (broad sense) were obtained for all growth related traits of turf grasses high genotypic and phenotypic coefficient of variation were recorded for shoot intensity (GCV=111.20, PCV=111.65) followed by leaf width (GCV=82.90, PCV= 83.77) and days to new growth (GCV=71.60, PCV=72.60). The maximum and minimum cluster mean value was observed for fresh weight and vertical leaf growth among all three clusters, respectively. High genetic advance was found for all characters, except monthly colour rating and turf quality. On basis of D^2 analysis, the thirteen genotypes were grouped into three clusters. The cluster I was very large and comprised of 9 genotypes and cluster II had 3 genotypes. While, cluster III was comprised only single genotype. The clustering pattern showed that genotypes of different geographical areas were clubbed in one group and also the genotypes of same geographical area were grouped into same cluster as well as in different cluster indicating formal relationship between geographical diversity and genetic diversity.

Keywords: Turfgrass, genetic diversity, D^2 analysis, GCV, PCV

Introduction
Turf is one of the blessings of nature and includes both service and beauty, a concept which originated when man started to domesticate animals (Huffine and Grau) [5]. It is considered as integral part of landscape ecological systems worldwide which provide aesthetic value Roberts *et al.,* [14]. All grasses are members of a single family called the Poaceae. However, only about 45 species among them are suited for turf use (Turgeon) [15]. Turf grasses consist of a remarkably diverse group of species which are selectively used on the basis of applications and or climatic conditions; cultivated for ornamental decoration around us, for recreation in golf courses and sports fields, and for land coverage and protection. Turfgrass is maintained on lawns, estates, parks, golf courses, playing fields and public grounds. Since the establishment of turfgrass culture in India and other parts of the world, it's improvement has always been a central issue. As a result of the persistent efforts of breeders, significant achievements have been made in turfgrass breeding.

Genetic reconstruction of a plant type is required for developing desirable varieties by incorporating and improving yield components and adaption traits for which existence and exploitation of the genetic variability is essential (Lakshmana *et al.,*) [7]. The existence of variability is essential for resistance to biotic and abiotic factors as well as for wide adaptability in the genotypes. Knowledge of heritability and genetic advance of the character indicate the scope for the improvement through selection. Heritability tells us about the additive genetic variance and phenotypic variance (Nyquist) [10]. Heritability estimates along with genetic advance are normally more helpful in predicting the gain under selection then heritability estimates alone (Johnson *et al.,*) [6]. Several measures *viz.,* multivariate analysis, heterosis, combining ability, geo-graphical distances, *etc*. have been used to assess genetic diversity among the plant populations. Among the multivariate analysis, Mahalanobis's generalized distance estimated by D^2 statistics (Rao) [13] is a unique tool for discriminating population considering a set of parameters together rather than inferring from indices based on morphological similarities and polygenic relationship. Among the available methods of multivariate analysis, Mahalanobis D^2-analysis appears to be most suitable for divergence study because it permits precise comparison among all possible pairs of population in any given group before affecting actual crosses. Most of the breeding work on turf grasses has been done in foreign countries like USA, Australia, Japan, Singapore, etc. but these grass species and varieties have not proved suitable for Indian agro-climatic conditions because a variety bred under a specific climatic zone, may not necessarily perform well under other climatic zones. No systematic work has been carried out on the aspect of turf grass breeding in India yet, therefore, the present investigation was undertaken to evaluate the turfgrass species for various growth related qualitative and quantitative traits.

Materials and Methods

The experiment was carried out at Floriculture Research Farm, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari during 2014-15. The experiment was laid out in randomized block design with three replications. Only healthy uniform sprigs of 10-12 cm long stolons were planted at 5 cm × 5 cm spacing in gross plots having 1.5 mx1.5 m size with net plots of 1.0 m x 1.0 m. Experimental materials included seven *Cynodon dactylon* L. genotypes, one wild selection of *Cynodon dactylon* L. (unknown parentage), one *C. dactylon* L.× *C. transvaalenesis* 'Tifdwarf' hybrid, two different species of *Zoysia viz., Z. tenuifolia* and *Z. matrella*, and three other genus *Axonopus compressus, Stenotaphrum secundatum* and *Eremochola ophiuroides.* The plants were maintained under uniform cultural conditions.

The morphological traits *viz.*, canopy height, vertical leaf growth rate, culm length, fresh weight, dry weight of clipping yield, stolon internodal length, stolon diameter, leaf length, colour rating, aesthetic value and shoot density, turf quality, turfgrass intensity, weed population and chlorophyll content in leaf were recorded monthly and calculated average of all months. while leaf width, root length and fresh shoot: root ratio were recorded at the end of experiment. Days taken to new growth appeared in 50 per cent plants and days to establishment (cover 90 per cent area of net plot). The mean performance of turfgrass genotypes for twenty morphological traits was calculated by Microsoft office excel worksheet, 2010 version. The analysis of variance for each variable was done as per the procedure described by Panse and Sukhatme [12]. The genotypic and phenotypic coefficient of variation was calculated according to formula given by Burton and De Vane (1953). Heritability (broad sense) in per cent was estimated as per the formula given by Burton and Vane De [3] and Johnson *et al.*, [6]. Genetic advance and genetic gain were calculated as per the formula suggested by Lush [8] and Johnson *et al.*, [6]. The mean and standard error (SE) were worked out as per standard methods (Panse and Sukhatme, 1967) and coefficient of variation was calculated using the following formula: CV = S/X*100 where S is the standard deviation and X is the mean. The genetic divergence analysis was carried out using the Mahalanobis's D^2 statistics (Mahalanobis) [9] and genotypes were grouped in clusters according to Tocher's method as described by Rao [13]. The intra and inter cluster distances and variances were worked out as per method suggested by Gomez and Gomez (1983).

Results and Discussion

The analysis of variance showed highly significant differences among the genotypes for all the twenty characters (Table 1). This indicated that large variability existed among the thirteen genotypes and that further analysis of genetic divergence is reasonable. The phenotypic coefficient of variation was observed to be higher than genotypic which indicates greater genotype-environment interaction (Table 2). However, high genotypic and phenotypic coefficient of variation were recorded for shoot intensity (GCV=111.20, PCV=111.65) followed by leaf width (GCV=82.90, PCV= 83.77) and days to new growth (GCV=71.60, PCV=72.60). It is reported that if the value of phenotypic coefficient of variation is greater than genotypic coefficient of variation, the apparent variation is not only due to genotype, but also due to influence of environment. High and moderate estimates of heritability (broad sense) were obtained for all growth related traits of turf grasses (Table 2). This suggested the scope of improvement of these traits through direct selection. Similar findings of high heritability for various turf-type characteristics were also recorded by Browning and Riordan [2] in Buffalo grass. Heritability along with genetic advance is useful in prediction in the selection of individual Johnson *et al*., [6]. High estimates of heritability with high to moderate estimates of genetic advance recorded for all the characters where careful selection may lead towards improvement for these traits. Hence, provides better opportunities for selecting plant material for these traits. High genetic advance was found for all characters, except monthly colour rating and turf quality. These findings revealed that genotypic variation studied for said traits was probably due to high additive gene effect Panse, [11]. Selection for these traits would, therefore, be effective when basis on phenotypic performance (Table 2).

On basis of D^2 analysis Mahalanobis, [9], the thirteen genotypes were grouped into three clusters (Table 3). The cluster I was very large and comprised of 9 genotypes and cluster II had 3 genotypes. While, cluster III was comprised only single genotype. The clustering pattern showed that genotypes of different geographical areas were clubbed in one group and also the genotypes of same geographical area were grouped into same cluster as well as in different cluster indicating formal relationship between geographical diversity and genetic diversity.

Intra and inter cluster distance (D) were computed for three clusters and presented in Table 4. The maximum intra cluster distances was observed in cluster III (174.64) indicating that differences in genotypes within the cluster and minimum intra cluster distances was observed in and cluster II (33.18) indicating that close resemblance between genotypes presented in this cluster. Inter cluster distance was maximum (174.64) between cluster II and III, while it was minimum (78.55) between cluster I and II.

The selection of genotypes based on cluster mean values for the better exploitation of genetic potential. Cluster means for different characters revealed that the cluster value excelled in respect of different characters (Table 5). Genotypes showed maximum and minimum value for fresh weight of clipping yield and vertical leaf growth rate in all cluster mean, respectively.

The selection and choice of parents mainly depend upon contribution of characters towards divergence (Table 6). The maximum contribution in the manifestation of genetic divergence was exhibited by shoot density, leaf width, chlorophyll, days to establishment, vertical growth, turfgrass intensity, internodal diameter and canopy height suggesting scope for these characters may be rewarding.

In the study, thirteen different genotypes of turfgrass were grouped into three clusters and suitable diverse genotypes were selected based on their cluster mean superiority and *per se* performance for different characters (Table 7). The species namely *C. dactylon* L. 'Local' was better for days to new growth, days to establishment, chlorophyll content, shoot root ration and root depth which deriving from cluster I. For other characters like aesthetic value, fresh and dry weight of clipping yield, stolon diameter, colour rating, turf quality and turfgrass intensity were better convenience to *C. d.* X *C. t.* ‘Tifdwarf' which derived from cluster I. The *Zoysia tenuifolia* was good performed for characters *viz.*, canopy height, culm length, vertical leaf growth rate, leaf width, stolon internodal length and shoot density (cluster-II). Likewise, *Stenotaphrum secundatum*. was good for weed count transform (Cluster-I).

Table 1: Analysis of variance for twenty traits in thirteen genotypes of turfgrasses

Source of variation	D. F.	Aesthetic value (1-5 point scale)	Canopy height (cm)	Culm length (cm)	Dry clipping yield (g m^{-2})	Fresh clipping yield (g m^{-2})	Stolon diameter (mm)	Leaf length (cm)	Monthly colour rating (1-9 scale)	Turf quality (1-9 scale)	Stolon internodal length (cm)
Replication	2	0.03	0.3374	0.79	5.136	129.52	0.0004	0.04	0.82**	0.09	0.02
Genotypes	12	1.69**	25.67**	47.87**	1315.12**	14569.04**	1.03**	8.31**	1.14**	1.49**	1.76**
Error	24	0.0301	0.15	0.21	7.95	58.48	0.001	0.03	0.11	0.08	0.01
S.Em ±	-	0.096	0.21	0.23	1.56	4.24	0.019	0.10	0.18	0.15	0.06
C.D at 5%	-	0.20	0.44	0.52	3.22	8.76	0.04	0.21	0.37	0.31	0.13
C.D at 1%	-	0.27	0.60	0.71	4.37	11.86	0.05	0.28	0.50	0.42	0.18

Source of variation	D. F.	Turf intensity (1-5 scale)	Vertical leaf grow rate (mm day^{-1})	Weed population count (25 m^{-2})	Days to new growth (50%)	Total chlorophyll (mg g^{-1})	Days to establishment (90%)	Root depth (cm)	Shoot :root ratio	Shoot density (count 25 cm^{-2})	Leaf width (mm)
Replication	2	0.06	0.001**	0.31	9.77	0.019	25.46	16.00	0.39	177.36*	0.17
Genotypes	12	1.54**	0.026**	57.00**	275.38**	1.16**	1798.28**	66.38**	1.28**	19462.23**	16.53**
Error	24	0.04	0.0001	2.22	3.96	0.009	16.07	6.25	0.27	52.22	0.11
S.Em ±	-	0.12	0.006	0.83	1.10	0.052	2.22	1.39	0.29	4.01	0.19
C.D at 5%	-	0.24	0.012	1.70	2.28	0.11	4.58	2.86	0.59	8.27	0.39
C.D at 1%	-	0.33	0.016	2.31	3.09	0.15	6.22	3.88	0.80	11.21	0.53

** Significant at P=0.01

Table 2: Range, mean and genotypic and phenotypic co-efficient of variation, heritability, genetic advance as per cent of mean for twenty traits in thirteen genotypes of turfgrasses

Sr. No	Characters	Range	Mean	GCV%	PCV%	Heritability (broad sense %)	Genetic advance % of mean
1.	Aesthetic value (1-5 point scale)	2.00-4.16	3.12	23.86	24.49	94.85	47.87
2.	Canopy height (cm)	4.47-13.59	7.75	37.62	37.94	98.30	76.83
3.	Culm length (cm)	2.19-14.56	8.46	47.12	47.43	98.71	96.44
4.	Dry clipping yield (g m^{-2})	15.25-92.64	58.73	35.53	35.86	98.21	72.55
5.	Fresh clipping yield (g m^{-2})	50.27-311.80	191.65	36.28	36.50	98.81	74.31
6.	Stolon diameter (mm)	0.39-2.55	0.92	62.62	62.73	99.67	128.80
7.	Leaf length (cm)	2.38-6.99	4.41	37.67	37.90	98.76	77.12
8.	Monthly colour rating (1-9 point scale)	5.87—8.07	6.99	8.39	9.59	76.71	15.14
9.	Turf quality(1-9 point scale)	6.28-8.30	7.04	9.75	10.51	86.21	18.66
10.	Stolon internodal length (cm)	1.17-3.33	2.16	35.38	35.78	97.81	72.09
11.	Turf intensity (1-5 point scale)	2.43-4.56	3.46	20.39	21.32	91.45	40.17
12.	Vertical leaf grow rate (mm day^{-1})	0.05-0.35	0.16	35.38	35.78	97.81	72.09
13.	Weed population (count m^{-2})	4.53-19.13	11.40	37.47	39.68	89.18	72.89
14.	Days to new growth (50%)	14.0-29.67	13.38	71.06	72.60	97.69	66.45
15.	Total chlorophyll (mg g^{-1})	0.71-2.84	1.90	32.64	33.02	97.69	66.45
16.	Days to establishment (90%)	16.33-83.33	39.00	62.49	63.34	97.37	127.04
17	Root depth (cm)	9.67-24.50	16.57	27.01	30.94	76.20	48.57
18	Shoot :root ratio	1.53-3.86	3.07	18.90	25.30	55.83	29.09
19	Shoot density (count 25 cm^{-2})	8.30-227.14	72.33	111.20	111.65	99.20	228.15
20	Leaf width (mm)	1.05-8.38	2.82	82.90	83.77	97.98	169.02

Table 3: The distribution of thirteen genotypes of turf grasses into three different clusters on the basis of Mahalanobis D^2 Statistic.

Cluster	No of genotypes	Genotypes
I	9	*Cynodon dactylon* L. ‘Panama’, *C. dactylon* L. ‘Palma’, *C. dactylon* L. ‘Panam’, *C. dactylon* L. ‘Bargusto’, *C. dactylon* L. ‘Black Jack’, *Stenotaphrum secundatum, C. dactylon* L.’Local’, *C. d.* X *C. t.* ‘Tifdwarf, *C. dactylon* L. ‘Selection 1’
II	3	*Axonopus compressus, Zoysia tenuifolia, Eremochola ophiuroides*
III	1	*Zoysia matrella*

Table 4: Average Intra and Inter – cluster (D^2) value for thirteen genotypes of turf grasses

Cluster	I	II	III
I	40.73	78.55	112.98
II		33.18	174.64
III			0.00

Table 5: Cluster mean for twenty characters in thirteen turfgrass genotypes.

Cluster Number	Aesthetic value (1-5 point scale)	Canopy height (cm)	Culm length (cm)	Dry clipping yield (g m^{-2})	Fresh clipping yield (g m^{-2})	Stolon diameter (mm)	Leaf length (cm)	Monthly colour rating (1-9 scale)	Turf quality (1-9 scale)	Stolon internodal length (cm)
I	2.85	8.91	10.42	64.79	209.71	0.92	4.95	6.84	6.81	2.31
II	3.86	5.15	3.23	42.97	140.81	0.44	2.49	7.69	7.94	1.29
III	3.37	5.19	6.44	51.51	181.67	2.55	5.34	6.23	6.47	3.34

Table 6: Per cent contribution of twenty characters towards genetic divergence in turfgrass

Sr. No	Character	Per cent contribution
1.	Aesthetic value (1-5 point scale)	0.00
2.	Canopy height (cm)	1.28
3.	Culm length (cm)	0.00
4.	Dry clipping yield (g m^{-2})	0.00
5.	Fresh clipping yield (g m^{-2})	0.00
6.	Stolon diameter (mm)	1.28
7.	Leaf length (cm)	0.00
8.	Monthly colour rating (1-9 point scale)	0.00
9.	Turf quality(1-9 point scale)	0.00
10.	Stolon internodal length (cm)	0.00
11.	Turf intensity (1-5 point scale)	1.28
12.	Vertical leaf grow rate (mm day^{-1})	1.28
13.	Weed population (count m^{-2})	0.00
14.	Days to new growth (50%)	0.00
15.	Total chlorophyll (mg g^{-1})	6.41
16.	Days to establishment (90%)	1.28
17	Root depth (cm)	0.00
18	Shoot :root ratio	6.41
19	Shoot density (count 25 cm^{-2})	67.95
20	Leaf width (mm)	12.82

Table 7: Diversity of turfgrass genotypes based on genetic distance and superior *per se* performance for the traits under investigation.

Sr. No	Character	Clusters	Parents	Mean
1.	Aesthetic value (1-5 point scale)	I	*C. d.* X *C. t.* 'Tifdwarf	4.16
2.	Canopy height (cm)	II	*Zoysia tenuifolia*	4.47
3.	Culm length (cm)	II	*Zoysia tenuifolia*	2.19
4.	Dry clipping yield (g m^{-2})	I	*C. d.* X *C. t.* 'Tifdwarf	92.64
5.	Fresh clipping yield (g m^{-2})	I	*C. d.* X *C. t.* 'Tifdwarf	311.80
6.	Stolon diameter (mm)	I	*C. d.* X *C. t.* 'Tifdwarf	0.39
7.	Leaf length (cm)	II	*Zoysia tenuifolia*	2.38
8.	Monthly colour rating (1-9 point scale)	I	*C. d.* X *C. t.* 'Tifdwarf	8.07
9.	Turf quality(1-9 point scale)	I	*C. d.* X *C. t.* 'Tifdwarf	8.30
10.	Stolon internodal length (cm)	II	*Zoysia tenuifolia*	1.17
11.	Turf intensity (1-5 point scale)	I	*C. d.* X *C. t.* 'Tifdwarf	4.56
12.	Vertical leaf grow rate (mm day^{-1})	II	*Zoysia tenuifolia*	0.05
13.	Weed population (count m^{-2})	I	*Stenotaphrum secundatum*	4.53
14.	Days to new growth (50%)	I	*C. dactylon* L.'Local	2.67
15.	Total chlorophyll (mg g^{-1})	I	*C. dactylon* L.'Local	2.84
16.	Days to establishment (90%)	I	*C. dactylon* L.'Local	14.00
17	Root depth (cm)	I	*C. dactylon* L.'Local	24.50
18	Shoot :root ratio	I	*C. dactylon* L.'Local	3.86
19	Shoot density (count 25 cm^{-2})	II	*Zoysia tenuifolia*	227.14
20	Leaf width (mm)	II	*Zoysia tenuifolia*	1.05

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