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GENETIC VARIABILITY, CHARACTER ASSOCIATION AND DIVERSITY AMONG MORPHOLOGICAL TRAITS IN CERTAIN GRASS SPECIES UNDER TROPICAL CONDITIONS

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Abstract

Morphological characterization of 12 grass genotypes using 10 traits was done in field experiment in a Randomized Block Design at the Horticultural College and Research Institute at Coimbatore for one years to examine the nature and magnitude of variability, heritability (broad sense) and genetic advance, correlation and diversity. All the traits exhibited high phenotypic co-efficient of variations (PCV) and genotypic co-efficient of variations (GCV) except the trait root density exhibited moderate. High heritability in conjugation with high genetic advance as percentage of mean (GAM) was absorbed for all the traits. Cluster analysis was carried out and nine clusters were obtained. Cluster I recorded the highest with three genotypes followed by Cluster II with two genotypes and remaining with one each. Significant and positive correlation was absorbed in all the traits. Significant and negative correlation was absorbed in the traits shoot density, shoot length, leaf length, leaf width, number of leaves per node.

Key words: Variability, heritability, correlation, Cluster analysis, grass and morphological.

Introduction

The current interest today in native plants has resulted in a public desire for more knowledge and potential uses of native plants in the landscape. The excitement with native plants in general has also ken extended to native grasses as well. Native grasses have tremendous potential for ornamental, reclamation and low-maintenance sustainable lawns. A recent search of the literature indicated that there has been very little research into the suitability of native grasses for turf grass use. Plant breeders have been evaluating and developing native and introduced grasses for use as low-input turf. The traditional turf grasses are better adapted to high input areas, whereas native grasses perform better under lower traffic and at higher mowing heights (Johnson, 2008).

Native species should be exploited in breeding programs for their adaptation to a broad range of soil and climate conditions (Willms *et al*., 2005) and their ability to withstand heat and drought stress with fewer irrigation needs (Johnson, 2000). Native accessions being developed as turf cultivars must also demonstrate limited growth, fine textured leaves, and quick recovery to damage from traffic and wear (Romani *et al*., 2002). It is important to evaluate the presence of genetic variability in the base population before initiating a selection program, as limited variability will lead to less significant gains over time (Surprenant and Michaud, 1988). Understanding the phenotypic variation of morphological and agronomic traits within a breeding population is crucial to the plant breeder in determining the potential application of the material, such as for turf (Wright *et al*., 1983). Genetic variation and heritability estimates help predict the response to selection for desired traits (Dudley and Moll, 1969). Selecting for characteristics with high broad-sense heritability will lead to faster and increased gains in the offspring than when selecting for traits with low heritability (Browning *et al*., 1994).

Considering the importance of researchable issues

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in grass, the study was carried out with the following objectives:

1- To assess the morphological diversity among the different grass species,

2- To study the association among different morphological attributes of the twelve grass species and

3- To study the genetic variability among the different grass species.

Materials and Methods

Field experiment was conducted at the Botanic Garden, Department of Floriculture and Landscaping, Horticulture College and Research Institute, TNAU, Coimbatore which is geographically situated at an altitude of 426.72 meters above mean sea level (MSL) and between 11°02" North latitude and 76°57" East longitude. The experiment was laid out in Randomized Block Design (RBD) with four replicates. Planting was done by sprigging method with plot size of 1 × 1 m^2.

Twelve genotypes collected from Coimbatore region and other private nurseries were evaluated. These genotypes are *Axonopus compressus*, *Brachiaria reptans*, *Digitaria bicornis*, *Cenchrus ciliaris*, *Cynodon dactylon X Cynodon transvaalensis*, *Dactyloctenium aegyptium*, *Ophiopogon japonicus*, *Paspalum vaginatum*, *Stenotaphrum secundatum*, *Stenotaphrum secundatum* 'Variegata', *Zoysia japonica* and *Zoysia tenuifolia*. Morphological data were collected for 10 characters. Data were collected on the following attributes - Shoot Length (SL), Shoot Density (SD), Leaf Length (LL), Leaf Width (LW), Internodal Length (IL), Number of Leaves $nodes^{-1}$ (NL), Number of Nodes per 10 cm^2 (NN), Root Density (RD), Root Length (RL) and Number of roots per 10 cm^2 (NR).

Tools of Functional Analysis

Estimation of Genetic Parameters

Genetic parameters like variability, GCV, PCV, heritability, genetic advance and genetic advance as per cent of mean were calculated by adopting the following formula

Genetic variability

a) Genotypic and phenotypic variances

The genotypic and phenotypic variances were calculated as suggested by Johnson *et al.* (1955).

$$\text{Genotypic variance } (s^2g) = \frac{M_1 - M_2}{r}$$

Where,

M_1 = Mean sum of squares for genotypes

M_2 = Mean sum of squares for error

r = Number of replications

Phenotypic variance $(\sigma^2p) = (\sigma^2g + \sigma^2e)$

Where,

σ^2e = Error variance

b) Phenotypic and genotypic coefficients of variation (PCV and GCV)

The method stipulated by Burton (1952) was used to calculate these parameters.

$$\text{PCV} = \frac{\sqrt{\sigma^2 p}}{general mean} \times 100$$

$$\text{GCV} = \frac{\sqrt{\sigma^2 g}}{general mean} \times 100$$

Where,

σ^2p = Phenotypic variance

σ^2g = Genotypic variance

The PCV and GCV were classified as per Sivasubramanian and Menon (1973).

Less than 10 percent = Low

10-20 percent = Medium

More than 20 percent = High

Heritability

Heritability (h^2) was computed following the method of Lush (1940) and expressed in per cent.

$$\text{Heritability} = \frac{Genotypic\ variance}{Phenotypic\ variance} \times 100$$

The heritability per cent was categorized as suggested by Robinson *et al.,* (1949).

0 to 30 percent - Low

31 to 60 percent - Moderate

Above 60 percent - High

Simple correlation co-efficients

Analysis of co-variance was done similar to that of analysis of variance taking two characters at a time. These were carried out with all possible combinations and the mean sum of product for genotypes; replications and error were worked out. The variance and co-variance components were utilized to calculate phenotypic and genotypic correlation co-efficients.

Genotypic co-variance (CoVg) = $\frac{MSP_1 - MSP_2}{ms}$

Where,

MSP_1 - Mean sum of products for genotypes

MSP_2 - Mean sum of products for error

ms - Number of replications

Phenotypic covariance (CoV P) = Genotypic co-variance + Error co-variance

From these co-variance components, genotypic and phenotypic correlation coefficients were worked out according to Al-Jibouri *et al.* (1958).

Genetic divergence studies - D^2 analysis

It is one of the potent techniques of measuring genetic diversity in plant breeding. The quantitative measurement of genetic divergence among the genotypes can be found out by D^2 Mahalanobis statistics (Rao, 1952). The steps involved in the analysis are,

a) Test of significance of difference by Wilk's statistic for aggregate traits.

b) Transformation of correlated variables

In terms to variance and covariances, the D^2 value was obtained by,

$D^2 = w^{ij} (x_i^1 - x_i^2) (x_j^1 - x_j^2)$

w^{ij} is the inverse of estimate of variance and covariance matrix. Transformation was done by pivotal condensation method. Transformation of correlated variables in to uncorrelated variables was done by substituting these values of x_1, x_2, x_3 and x_4 in the transformed equation and the corresponding transformed values y_1, y_2, y_3 and y_4 were obtained (original mean to transformed data).

c) Computation of D^2 values

For each combination of population, the mean of deviation for the characters was computed and the D^2 was calculated as the sum of squares of these deviations.

i.e,

$D^2 = (y_i^1 - y_i^2)^2$

Where i = 1, 2, 3….. p characters

d) Test of Significance of D^2 values

The significance of D^2 values for a pair of population was tested against the table values of χ^2 for 'p' degrees of freedom.

Where p = Total number of characters

e) Grouping into clusters

The method suggested by Tocher (Rao, 1952) was followed for cluster formation. The accessions were arranged in the order of their relative distance from each other.

The values were arranged in the ascending order of magnitude in each column.

Two genotypes having smallest distance from each other were considered first to which a genotype having smallest average D^2 value from the first two genotypes was added. If at any stage, the average D^2 of a group appeared to be high from those already included, it was considered that the group does not fit with the former cluster and hence taken outside the first cluster and the second cluster was formed. This process was continued and clusters were formed.

f) Estimation of average intra cluster distances

The average of the distances of all possible combinations of accessions included in a cluster was calculated.

g) Estimation of average inter cluster distances

This was calculated by measuring the distances between the clusters. The clusters were taken one by one and their distance from each other was calculated.

ni = Number of genotypes in cluster i

nj = Number of genotypes in cluster j

h) Contribution of individual characters towards divergence

In all the combinations, each character was ranked on the basis of $di = y_i^1 - y_i^2$ values. Rank one was given to the highest mean difference and the rank 'p' to the lowest mean difference where 'p' is the total number of character. The number of times appearing first in ranking for each character was counted and the per cent contribution was calculated taking the total number of combinations as 100.

Results

Heritability Estimates: Estimates of phenotypic, genotypic and environmental variances for all attributes are shown in table 1. The genotypic variance was higher than the environmental variance in all the traits studied. Estimates of the phenotypic and genotypic coefficient of variability and the difference between them are shown in table 2. The highest PCV and GCV estimates obtained were for number of leaves per node. Estimates of heritability and expected genetic advances as percentages of the general mean are also shown in table 2. Heritability estimate highest for all the traits studied.

Interrelationship between attributes

The simple correlation coefficient between attributes studied is shown in table 3. Shoot length had significant and positive correlation with shoot density ($r = 0.36$, $p < 0.05$), leaf width ($r = 0.42$, $p < 0.01$), root length ($r = 0.45$, $p < 0.01$), leaf length ($r = 0.47$, $p < 0.01$) and internodal length ($r = 0.79$, $p < 0.01$). The character shoot length had significant and negative correlation with number of nodes ($r = -0.39$, $p < 0.05$). The character shoot density recorded significant and positive correlation with internodal length ($r = 0.52$, $p < 0.01$), number of nodes ($r = 0.45$, $p < 0.01$), root density ($r = 0.37$, $p < 0.05$), root length ($r = 0.33$, $p < 0.05$) and number of roots ($r = 0.52$, $p < 0.01$). The character shoot density had significant and negative correlation with number of leaves per node ($r = -0.79$, $p < 0.01$).

The character internodal length recorded significant and positive correlation with root length ($r = 0.51$, $p < 0.05$). The character internodal length had significant and negative correlation with number of nodes ($r = -0.38$, $p < 0.05$). In the grass species, the character number of nodes had significantly and positive correlation with root density ($r = 0.62$, $p < 0.01$) and number of root ($r = 0.67$, $p < 0.01$). Root density had significant and positive correlation with number of root ($r = 0.82$, $p < 0.01$).

Cluster analysis

The analysis of morphological diversity through the cluster analysis has been shown in table 4. By the application of clustering technique twelve grass species were grouped into 9 clusters. Among the 9 clusters, cluster I was the largest with three grass species followed by cluster II constituting two grass species. Cluster III-IX composed of one species each.

The intra and inter cluster D^2 values among the nine clusters are presented in table 5. Intra cluster distance ranged from 0.00 to 7837.51. Cluster II recorded the maximum intracluster distance of (7837.51) followed by Cluster I (6135.23). The maximum intercluster distance were between cluster VII and IX (32012.24), followed by V and IX (26438.69).

Table 1: Estimates of phenotypic, genotypic and environmental variances for different attributes.

Attributes	Phenotypic variances	Genotypic variances	Environmental variances
Shoot length	288.502	287.069	1.433
Shoot Density	268.767	262.919	5.848
Leaf length	6.165	6.093	0.073
Leaf width	0.108	0.107	0.001
Internodal length	6.950	6.924	0.026
No of leaves per node	15.823	15.805	0.019
No of nodes per 10 cm^2	12.863	12.834	0.029
Root density	1.076	1.028	0.048
Root length	12.278	12.083	0.196
Number of roots per 10 cm^2	5038.333	5018.332	20.001

Table 2: Estimates of phenotypic (PCV) and genotypic (GCV) coefficients of variability, heritability (h^2) and expected genetic advance as percentage of mean (GAM) from the means of grass attributes.

Traits	PCV	GCV	PCV-GCV	h^2 (%)	GAM
Shoot length	70.10	69.93	0.17	99.50	143.70
Shoot density	23.76	23.50	0.26	97.82	47.89
Leaf length	37.75	37.53	0.22	98.82	76.86
Leaf width	53.94	53.80	0.14	99.48	110.54
Internodal length	86.39	86.23	0.16	99.62	177.30
No of leaves per node	154.08	153.99	0.09	99.88	317.03
No of nodes per 10 sq.cm	66.25	66.18	0.07	99.77	136.17
Root density	16.09	15.73	0.36	95.49	31.66
Root length	30.14	29.89	0.25	98.41	61.10
Number of roots per 10 cm^2	50.36	50.26	0.16	99.60	103.34

The relative contribution of each character towards genetic divergence is presented table 6. Root number (53.03 percent) contributed maximum towards genetic divergence followed by leaf width and root density (13.64 per cent), root length (7.58 percent).

The nine cluster means for the ten characters are given in table 7. Cluster III recorded the highest mean value for root number (256.28) followed by cluster IX (250.63). The lowest mean value (0.14) for the character leaf width was recorded by cluster II followed by clusters III (0.35).

Discussion

High PCV and GCV were recorded for the traits of shoot length, shoot density, leaf length, leaf width, internodal length and number of leaves per node, number of nodes per 10 cm^2, root length and number of roots table 2. Medium PCV and GCV were recorded for the trait root density. Earlier workers have also reported similar trends in plants belonging to poaceae. Geremew-gebeyhu (1993) recorded a wide range of variability for leaf and stem characteristics in sorghum. Idris (2006) reported highly significant differences for plant height, leaf area and number of leaves in sorghum. Bello *et al.,* (2007) also showed that genotypes of sorghum exhibited variability in plant height and number of leaves.

Johnson *et al*., (1955) suggested that heritability in combination with genetic advance was more effective and reliable in predicting the resultant effect of selection

Table 3: Simple Correlation Matrix between grasses attributes.

Character	*SL*	*SD*	*LL*	*LW*	*IL*	*NL*	*NN*	*RD*	*RL*	*NR*
SL	1.00	0.36*	0.47**	0.42**	0.79**	-0.25	-0.39**	-0.14	0.45**	-0.19
SD		1.00	-0.15	0.24	0.52**	-0.79**	0.45**	0.37*	0.33*	0.52**
LL			1.00	0.31*	0.63**	0.26	-0.73**	-0.68**	0.26	-0.73**
LW				1.00	0.46**	-0.11	-0.60**	-0.21	0.32*	-0.08
IL					1.00	-0.26	-0.38*	-0.16	0.51*	-0.12
NL						1.00	-0.41**	-0.35*	-0.47**	-0.27
NN							1.00	0.62**	-0.07	0.67**
RD								1.00	0.13	0.82**
RL									1.00	-0.06
NR										1.00

SL- Shoot Length	SD- Shoot Density	LL- Leaf Length	LW- Leaf Width
IL- Internodal Length	NL- Number of Leaves nodes^{-1}	NN- Number of Nodes per 10 cm^2	RD- Root Density
RL- Root Length	NR- Number of roots per 10 cm^2	*significant at 5 % level	**significant at 1 % level

than heritability alone. High value of heritability together with high genetic advance for any character indicates additive gene action and selection will be rewarding for improvement of such traits. In the present study, high heritability coupled with high GAM has been recorded for all the traits table 2. High heritability estimates have also been observed for plant height measurements in Kentucky bluegrass (Pepin and Funk, 1974) and maize (*Zea mays* L.) (Soleri and Smith, 2002). Greene (2007) stated that broad sense heritability was high for internode length, leaf length and leaf width and the genetic variance was twice the genotype×year variance for leaf length and several times more for internode length and leaf width. These traits influence the plant architecture and are related to overall density and turf quality. In the present study also, the similar findings was observed.

Table 4: Cluster composition of 12 grass species based on morphological characters.

Cluster number	No. of Genotypes	Genotypes
I	3	*Axonopus compressus, Ophiopogon japonicus* and *Paspalum vaginatum*
II	2	*Zoysia japonica* and *Zoysia tenuifolia*
III	1	*Cynodon dactylon* x *Cynodon transvaalensis*
IV	1	*Dactyloctenium aegyptium*
V	1	*Stenotaphrum secundatum*
VI	1	*Stenotaphrum secundatum* 'Variegata'
VII	1	*Digitaria bicornis*
VIII	1	*Cencrus ciliaris*
IX	1	*Bracharia reptans*

In the present study, significant and positive correlation were found for the trait shoot length with shoot density, leaf width, leaf length, internodal length and root length table 3. However, these results are in contrary with those of Pooran and Chard (2000). The character shoot length had significant and negative correlation with number of nodes table 3. The trait shoot density recorded significant and positive correlation with internodal length, number of nodes, root density, root length and root number.

The results of the present study are in line with earlier reports. Leaf width has been shown to be correlated with shoot density (Turgeon, 1999). Thereby, plants that have a high shoot density also have finer leaves and plants with low shoot density have wider leaves (Nilsen and Orcutt, 1996). The character number of nodes had significant and positive correlation with root density and

Table 5: Intra and inter cluster averages of D^2 values among 9 clusters of grass species

Cluster	I	II	III	IV	V	VI	VII	VIII	IX
I	6135.23	7810.71	12214.24	7416.71	11979.63	10089.35	15951.21	11906.71	17728.57
II		7837.51	11034.77	10852.74	15581.62	14148.92	20685.97	11226.97	19798.95
III			0.00	9696.71	17721.27	16483.31	22929.34	17397.74	21354.16
IV				0.00	20383.66	14618.39	25727.244	9944.61	18381.82
V					0.00	12243.16	22817.29	15260.44	26438.69
VI						0.00	17926.61	13146.49	23330.84
VII							0.00	19810.28	32012.24
VIII								0.000	23710.16
IX									0.000

Table 6: Contribution of characters to genetic divergence in grass species.

Sl. No.	Characters	Number of times Ranked First	Contribution percentage(%)
1.	Shoot length	0	0.00
2.	Shoot density	1	1.52
3.	Leaf length	1	1.52
4.	Leaf width	9	13.64
5.	Internodal length	2	3.03
6.	No. leaves per node	2	3.03
7.	No. of nodes per 10 sq.cm	2	3.03
8.	Root density	9	13.64
9.	Root length	5	7.58
10.	Number of root per 10 sq.cm	35	53.03

number of roots. Root density had significant and positive correlation with number of roots. Deeper rooting was found to be highly correlated with greater turf quality in many other grasses like *Cynodon spp*. (Hays *et al.,* 1991), Zoysia spp. (Marcum *et al*., 1995) and Festuca spp. (Qian *et al*., 1997).

Among the nine clusters, cluster I was the largest with three genotypes followed by cluster II constituting two genotypes table 4. Clusters III-IX composed of one accession each. As opined by Murthy and Arunachalam (1966), this non parallelism may be due to genetic drift and intense natural and human selection for diverse adaptive gene complexes under different environments, causing greater diversity among genotypes rather than their geographic distances.

However, several studies have reported a continuum of overlapping variation among the different species and difficulty of classifying the morphological intermediates (Yaneshita *et al*., 1997; Patton and Riecher, 2007; Tsuruta *et al.,* 2011; Schwartz *et al.,* 2010). Anderson (2000) reported on the ability to classify these morphological intermediates or possible interspecific hybrids with the aid of genetic analysis.

For ornamental crops such as turf grass, appearance and quality are usually of primary importance in breeding and selection and therefore are commonly collected in diversity assessments. These include leaf, internode and inflorescence traits as well as the plants overall growth habits (Hanna and Burton, 1978; Busey, 1986; Anderson, 2000; Liu *et al.,* 2003).

Morphological diversity was present within and among twelve grass species. Similar kind of findings were also reported by Hanna and Burton (1978); Hanna 1995; and Liu *et al*., 2003 on Centipede grass accessions. As diversity has been found to be high, the use of morphology to classify twelve grass species appears to be adequate. Extremely low morphological diversity among Centipede grass accessions were observed by Hanna and Burton (1978); Hanna 1995; and Liu *et al*., 2003.

Conclusion

A significant correlation of traits as obtained in this work showed that these characters could be improved simultaneously. High heritability of some traits indicated that these characters could be improved. The phenotypic variability and high heritability of the traits studied is manifested in high genetic advance in the traits exhibited in the genotypes. High heritability indicated a preponderance of additive gene effect and could be transferred to the progeny in F_1 hybrids.

High heritability estimates and high expected genetic advance could be used as selection criteria in early generation of test population. The breeder must however pay attention to the negative correlation that existed amongst the characters. The study showed genetic variability amongst the genotypes and this is important in selection of parent for hybridization. Since crop improvement depends upon magnitude of genetic variability in base population.

This study has quantified morphological variation in

Table 7: Cluster means of characters for twelve grass species.

Characters Cluster	Shoot length	Shoot density	Leaf length	Leaf width	Internodal length	No. of leaves node^{-1}	No. of nodes per 10 cm^2	Root density	Root length	Number of roots per 10 cm^2
I	9.97	54.97	7.42	0.61	1.74	6.22	3.59	5.64	9.94	99.70
II	8.60	72.51	3.75	0.14	0.95	1.00	11.38	7.50	10.29	194.99
III	25.43	85.43	5.03	0.35	2.71	2.01	10.05	7.54	9.55	256.28
IV	12.63	60.15	7.82	0.65	1.30	1.00	3.26	5.51	12.03	65.16
V	44.93	83.94	9.88	0.74	9.29	1.98	2.47	6.42	16.29	153.06
VI	49.99	79.20	9.90	0.89	6.73	1.98	3.47	4.95	9.90	69.30
VII	38.12	69.30	3.96	0.69	2.08	0.99	5.19	5.94	11.88	148.50
VIII	48.14	59.55	8.44	0.55	4.17	0.99	2.48	6.95	16.18	57.57
IX	24.46	80.20	4.01	1.03	3.21	1.00	4.51	8.02	13.23	250.63

different genotypes and the possible genetic control of such characters. These results will be highly valuable to botanists and breeders who need to understand and manipulate morphological characters in grass species. Future studies should examine reproductive, seed set and the genes involved in controlling inflorescence architecture.

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