Journal of Applied Horticulture, 9(2): 118-122, July-December, 2007

Comparative morphology and RAPD analysis of some turfgrass cultivars grown in Saudi Arabia

N.S. Al-Khalifah, E. Askari and A.E. Shanavaskhan*

King Abdulaziz City for Science and Technology (KACST), Post Box 6086, Riyadh 11442, Saudi Arabia.
**E-mail: shana_khan2000@yahoo.co.in*

Abstract

With the increasing number of turfgrass cultivars, development and use of reliable identification methods is becoming important. Random amplified polymorphic DNA (RAPD) markers along with morphological markers proved useful for cultivar identification. Seven turfgrass cultivars encompassing four bermudagrass and three zoysiagrasses were grown under uniform greenhouse conditions and their key diagnostic features were described. Bulk samples of leaves were collected from each cultivar and subjected to RAPD analysis using standard protocols. Out of the 35 Operon primers used, 20 detected polymorphism among the cultivars. 'Nagissa' and 'Miyako' zoysiagrasses showed close genetic relationship as compared to the rest of the cultivars. They had the highest value in the similarity matrix for Nei and Li's coefficient (0.802) while one variant of Miyako clustered with Bermuda-1. Tifgreen Bermuda and Bermuda-2 also clustered together while 'Tifway' stood apart. Analysis of the morphological data showed that the variant of ' Miyako' belonged to the *Zoysia* genus but its genetic affinity with Bermudagrass needs to be explained. Within and between species, the cultivars having similar leaf-texture showed a tendency to cluster together.

Key words: Miyako, Nagissa, Tifgreen, Tifway, Zoysiagrass, Bermudagrass, RAPD, morphology

Introduction

Turfgrasses have been the subject of conventional systematic studies using comparative morphological and ecological characters. However some turfgrasses have similar or narrow distinguishing morphological characters that complicate taxonomical classification and demand genetic evidence to prove phylogenetic relationships at the inter specific level (Caetano-Anolles,1998). Since morphological characters of the turfgrass, such as growth, leaf-colour, size, texture, internodal length etc., are highly variable depending on the climatic and edaphic changes, cultivars within some species are difficult to distinguish. In a desert country like Saudi Arabia, the environmental conditions are very harsh and extremely flexible during different seasons, and differs significantly from the other locations in which these turfgrasses are cultivated. Phenotypic expressions of some turfgrass cultivars in response to these harsh conditions complicate identification within the species and rarely between the cultivars of allied species. Okawara *et al*. (2002) reported various responses of some Zoysiagrasses and other grasses to the environmental conditions of Saudi Arabia. One of the solutions to overcome this complication is to grow all the cultivars under uniform environmental conditions and then describe the morphological characters.

Recent advances in technology have shown that amplification of DNA using single arbitrary primers generates an almost infinite number of polymorphisms (Williams *et al.*,1990; Caetano-Anolles *et al*.,1991). RAPD techniques were successfully utilized in identifying the cultivars of perennial ryegrass (Sweeny and Danneberger, 1994;1997), *Zoysia japonica* Steud (Caetano-Anolles *et al*.,1991), *Eremochloa ophiuroides* (Munro.) Hack.(Weaver *et al*.,1995), Kentucky Bluegrass (Ohumura *et al*.,1997), *Paspalum vaginatum* O.Swartz (Chen *et al*., 2005) and should prove useful for identifying cultivars in other turfgrass species.

The objective of this study was to identify the key diagnostic morphological features of 3 cultivars of zoysiagrass and 4 cultivars of bermudagrass grown under uniform greenhouse conditions and to apply RAPD techniques for the identification and determination of their phylogenetic relationships.

Materials and methods

Four cultivars of bermudagrass and three cultivars of zoysiagrass were procured from various sources (Table 1). Tillers of the cultivars were planted in polystyrene pots of 27cm diameter and 30cm height filled with peat moss, perlite and coarse sand in the ratio 2:1:1. All pots were nurtured in a greenhouse with temperature ranging between 28°C in day and 15°C during night and having a photoperiod of 14 hours. The pots were arranged in a randomized complete block design (RCBD) with three pots per cultivar as one replicate and four replicates were used.

After 3 months of growth, 10 randomly selected shoots were carefully removed from each replicate pot and all the replicates were represented. Three leaf samples were collected from the apical portion of each shoot, after leaving the terminal rolled ones. Length and maximum width of leaf samples were measured using a millimetre scale. The formula used to calculate leaf area was: leaf area = length x maximum width x 0.84 as reported by Shabana and Antoun (1980). Internodal length of each sample collected from each cultivar was also measured from the detached shoots, after leaving the terminal long ones. Measurements of leaf-length, width, internodal length and leaf-area are provided

Table 1. Name and source of turf grass cultivars

Sl. No	Name	Scientific name	Source
1	Miyako	*Zoysia matrella* (L.) Merr.	Saudi-Japan Research Project.
2	Nagissa	*Zoysia matrella* (L.) Merr.	Saudi-Japan Research Project.
3	Variant of Miyako	*Zoysia matrella* (L.) Merr.	Saudi-Japan Research Project.
4	Tifgreen	*Cynodon dactylon* (L.)Pers. x *C. transvaalensis* Burtt.-Davy	Southern Turf Nurseries, Georgia.
5	Tifway	*Cynodon dactylon* (L.) Pers. x *C. transvaalensis* Burtt.-Davy	
6	Bermudagrass (1)	*Cynodon dactylon* (L.) Pers.	Local farms (cultivated)
7	Bermudagrass (2)	*Cynodon dactylon* (L.) Pers.	Central part of Saudi Arabia (wild)

in Table 2. Inflorescences were collected during flowering and preserved in FAA and then studied under a stereomicroscope. Colour of the inflorescence, stamen, and stigma was recorded before preservation and measurements of diagnostic characters were taken. Leaf texture was evaluated visually on a 1-9 scale where 1-4=coarse, 5-7=medium and 8-9= fine textured.

Data were analyzed statistically by analysis of variance and least significant differences (LSD) according to Snedecor and Cochran (1973). Samples collected from three pots of each cultivar were treated as one replicate.

Composite samples of leaves were collected from each replicate pot of the seven cultivars for RAPD analysis. Total genomic DNA was extracted from the young leaves of each cultivar. The leaves were ground into a fine powder in liquid nitrogen using pestle and mortar and DNA was extracted following the protocol of Dellaporta *et al*. (1983). The quantity and quality of the DNA extracted was determined using a flourometer (Hoefer DyNA Quant 200; Pharmacia Biotech, Piscatawaym, N.J.). The stock DNA samples were diluted with sterilized distilled water to make a working solution of 10 ηg μL^{-1} for use in PCR analysis.

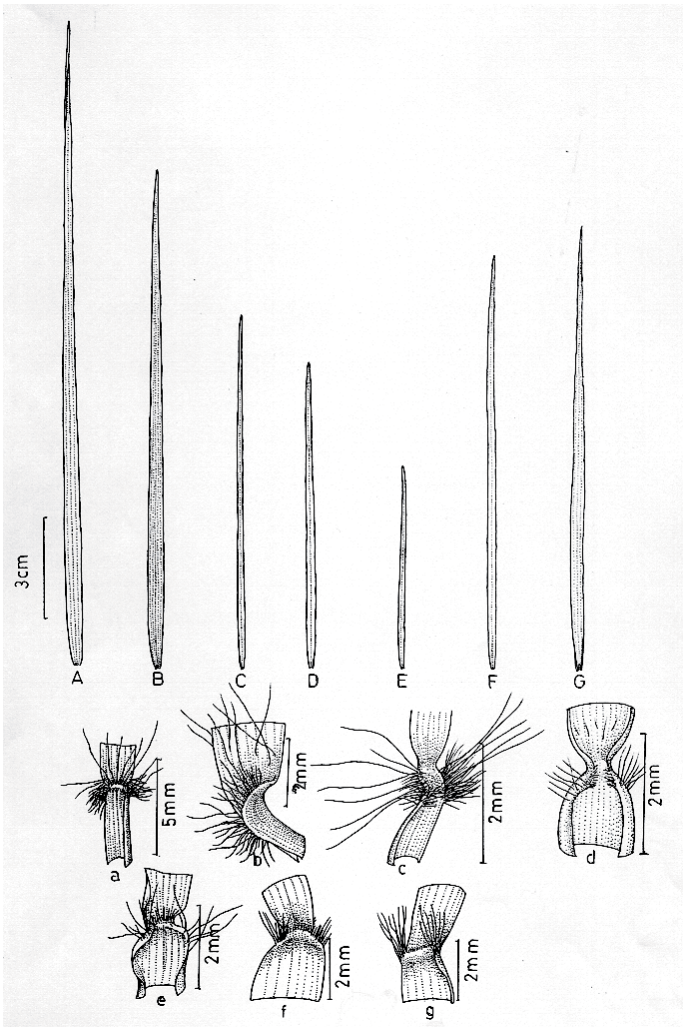

Fig.1. Leaves(A-G) and collar regions(a-g) of seven cultivars of turfgrass. A,a Miyako; B,b Variant of Miyako; C,c Nagissa; D,d Tifgreen; E,e Tifway; F,f Bermuda-1; G,g Bermuda-2.

Thirty five random 10-mer RAPD primers (OPERON Technologies, Alameda, Calif.) of A, B and C series were used for PCR amplification of the 7 DNA samples. The primers were diluted in TE buffer at a concentration of 10 ηg μL^{-1}.

PCR amplification reactions were performed in a Flexigene Thermal Cycler (Techne Inc.) in volumes of 25 µL containing 1 unit of *Taq* DNA polymerase (Amersham Pharmacia Biotech) per reaction. The amplified fragments were separated by electrophoresis according to their molecular weight in 1.4 % (w/w) agarose gels horizontally submerged in 1x TBE buffer. The gels were then stained with ethidium bromide (10 µg mL^{-1}) solution for 20 min. The RAPD products were observed on a UV transilluminator and documented by the Gel Documentation System of Bio Rad. (Hercules, Calif.). To estimate the molecular weight of the fragments λDNA Ladder (Sigma) was run in the gels as standard size marker.

The 20 selected amplification profiles of the 7 different turfgrass samples were compared with each other using 'Diversity Data Base' (Bio Rad) software. The data were applied to estimate the similarity on the basis of the number of shared amplified fragments (Nei,1978; Nei and Li, 1979). Cluster analysis by the unweighted pair group method of arithmetic means (UPGMA) was also performed using the 'Diversity Data Base' (Bio Rad) software.

Results and discussion

Morphology

Zoysiagrass: The Zoysiagrass samples were more compact in appearance with uniformly short internodes. Leaves are rolled in bud-shoot; ligules are a fringe of hairs longer than in the bermudagrasses. Inflorescence is a terminal unbranched spike-like raceme with many spikelets.

Miyako is a sod-forming cultivar of Zoysiagrass that possess both stolons and rhizomes. Leaf blades are 19 x 0.39 cm, smooth with occasional hairs near the base, margins entire and pointed at apex. Collar is 1.5mm wide with tuft of hairs at the margins (Fig. 1: A, a). Racemes 5 x 0.3 cm, with pedicelled, adpressed spikelets. Spikelets small, ovate-acuminate. Stamens yellow. Stigma long, brown and feathery.

Variant of Miyako is very much similar to Miyako except for its

short stature and very soft texture. Leaf blades are 14.5 x 0.38 cm, smooth, more hairy towards the base. Collar constricted, tuft of hairs present at the margins (Fig. 1: B, b). Racemes 4 x 0.4 cm; spikelets small, ovate-acute; stamens and stigma yellow.

Nagissa is a coarse textured, dark green cultivar of Zoysiagrass. Leaf blades are 10.3 x 0.17 cm, glabrous, entire, and pointed at apex. Collar narrow, not constricted (Fig. 1: C, c). Racemes 1.5 x 0.3 cm, brownish-pink. Spike let ovate-acute, rounded at base. Stamens yellow; stigma pink.

Analysis of the measurements of leaves and internodes showed that among Zoysiagrasses there were very distinct variations in the leaf-length and leaf-area but only Nagissa showed distinctiveness in leaf-width (Table 2). The leaf-area of Nagissa was significantly low but their internodal length showed no significant variations with the other zoysiagrass cultivars. Nagissa produced pink coloured short inflorescence while the other two cultivars produced yellow coloured, almost three times longer inflorescence than Nagissa. Regarding leaf-texture Miyako and Nagissa were coarse-textured while the variant of Miyako was soft-textured.

Bermudagrass: In the Bermudagrass samples, leaves are borne on stems with long internodes alternating with one or more very short internodes. Leaves are folded in bud-shoot; ligules are a fringe of short hairs. Inflorescence is 3-7 digitately arranged spikes from the top of a terminal stalk. Spikelets are similar in two rows on the abaxial side of a flattened axis.

Tifgreen is a low growing, rapid spreading cultivar of bermudagrass that develops a dense turf by producing stolons. Leaf blades are 9.05 x 0.27 cm, smooth, sparsely ciliate on the ventral side, glabrous on the dorsal side and sharply-pointed. Collar is continuous and has long, tufted hairs on the margins (Fig. 1: D, d). Inflorescence with 3 to 4 spikes, up to 2.8 cm. Stamens yellow. Stigma dark pink, giving pinkish appearance to the inflorescence.

Tifway is very similar to Tifgreen except for its greater stiffness of leaf blades and darker green colour. Persisting scales are very prominent at the nodes. Leaf blades are 5.6 x 0.14 cm, sharply pointed, ciliate on the ventral side, dorsal glabrous. Collar narrow, tuft of hairs up to 2 mm long (Fig. 1: E, e). Flowering is observed very rarely. Spikes, when present, 2-3 only.

Leaf blades of Bermudagrass-1 are 12 x 0.3 cm, pubescent, minutely serrulate on the margins, sharply pointed. Collar is constricted, narrow, glabrous and hairy on margins with a tuft of hairs of 2-5 mm long (Fig. 1: F, f). Inflorescence with 5 to 7 spikes. Spikelets 3 x 1.25 mm, ovate-acute and greenish-white in appearance. Stamens light yellow; stigma greenish-white, much branched.

Leaf blades of Bermudagrass-2 are 13x0.34 cm, smooth to sparsely pubescent and sharply-pointed. Collar is continuous with tuft of hairs at the margins (Fig. 1: G, g). The inflorescence consists of 3-5 spikes. Spikelets 2.5 x 1 mm, ovate-acute and dark pink in appearance.

Among bermudagrass cultivars Bermudagrass-1 was tall with significantly longer internodes and produced inflorescence with 5-7 spikes while the Bermudagrass-2 was short, and produced inflorescence with 3-5 spikes. Tifgreen and Tifway were quite distinguishable by their leaf-length, leaf-width, area and texture but they were not significantly different in their internodal length. Regarding leaf-texture, Tifgreen and Bermudagrass-2 were medium-textured while Tifway was coarse and Bermudagrass-1 was fine-textured.

RAPD analysis: Out of the 35 RAPD primers screened, 20 primers detected clear polymorphism between the genotypes and were found reproducible in repeated trials. All the seven cultivars revealed a unique profile with the 20 primers and thus selected for DNA-fingerprinting. Different levels of polymorphism were detected among the 7 cultivars with different primers (Fig. 2).

The similarity matrix based on Nei and Li's (1979) coefficient showed genetic distance ranged from 0.45 to 0.80 (Table 3). A dendrogram constructed by using unweighted paired group method of arithmetic means (UPGMA) showed maximum

Table 2. Measurements of leaf, internodes and texture of seven cultivars of turfgrass (Mean ± SD)

Name of cultivar	Leaf-length (cm)	Leaf-width (cm)	Area (cm^2)	Internodal length (cm)	Leaf-texture*
Miyako	19.16 ± 3.09	0.39 ± 0.02	6.33	5.16 ± 0.92	3.7
Variant of Miyako	14.65 ± 1.82	0.38 ± 0.06	4.73	4.61±1.01	8.0
Nagissa	10.38 ± 0.17	0.17 ± 0.01	1.49	2.80 ± 0.69	3.0
Tifgreen	9.05 ± 0.12	0.27 ± 0.01	2.10	5.87 ± 0.85	6.7
Tifway	5.62 ± 0.22	0.14 ± 0.01	0.67	3.67 ± 0.79	2.2
Bermudagrass-1	12.20 ± 0.51	0.29 ± 0.04	3.02	11.22 ± 1.21	8.0
Bermudagrass-2	13.09 ± 1.67	0.34 ± 0.02	3.73	6.97 ± 1.79	5.5
LSD (P<0.05)	2.58	0.04	0.62	3.18	1.3

*Leaf texture was determined visually with a scale of 1-4=coarse, 5-7=medium, 8-9=fine.

Table 3. Similarity matrix (Nei and Li's coefficients) of 7 turfgrass cultivars obtained from RAPD markers

		1	2	3	4	5	6	7	8
Bermudagrass 1	1	100.0							
Bermudagrass 2	2	50.3	100.0						
Miyako	3	59.6	52.3	100.0					
Nagissa	4	55.2	47.6	80.2	100.0				
Tifgreen	6	59.3	65.3	52.6	49.1	53.3	100.0		
Tifway	7	64.5	49.7	55.9	53.6	59.1	51.3	100.0	
V. Miyako	8	73.2	45.0	57.8	58.2	63.2	48.8	69.0	100.0

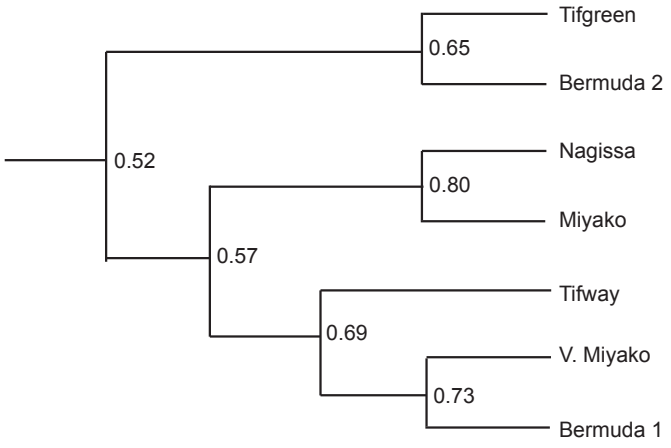

Fig. 3. A dendrogram of genetic relationship among seven cultivars of turfgrass based on the RAPD analysis.

similarity between the cultivars Nagissa and Miyako (Fig. 3). But variant of Miyako clustered with Bermuda-1, with the second highest value in the similarity matrix (0.73). Variant of Miyako showed 0.57 similarity matrix values with its other two Zoysiagrasses. The morphological analysis clearly indicated that the variant of Miyako, with rolled leaves in bud shoots; ligules with a fringe of long hairs and terminal unbranched spike like raceme belong to the *Zoysia* species. The unusual

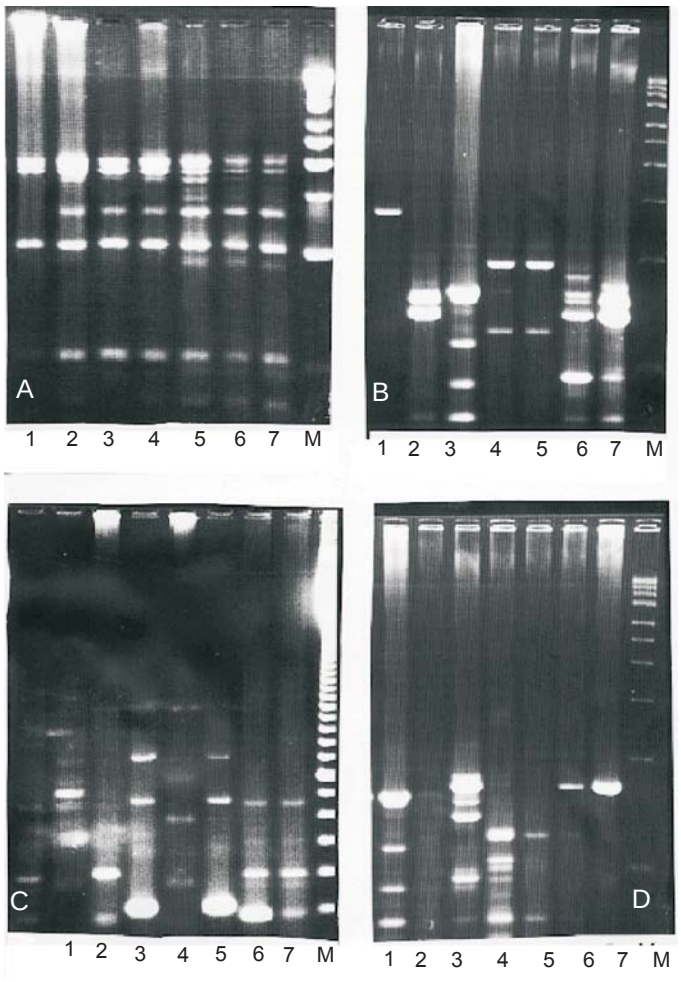

Fig. 2. (A-D) RAPD profiles of seven turfgrass cultivars using OPA11, OPA4, OPA6 and OPA14 primers, respectively. Bermudagrass 1, Bermudagrass 2, Miyako, Nagissa, Tifgreen, Tifway V. Miyako, molecular markers represent 1, 2, 3, 4, 5, 6, 7 and M lane, respectively

behaviour of the variant of Miyako, clustering with Bermudagrass cultivars needs to be explained. Stammers *et al*. (1995) analyzed phylogenetic relationships in the *Lolium/Festuca* complex using RAPD technique faced a similar problem in *Festuca pratensis* which, clustered with *Lolium multiflorum* and *L.perenne*. They assumed that the reason for this unusual behaviour of *Festuca pratensis* could be homoplasy or convergence effect. Tifgreen and Bermudagrass-2 were also closely related with the similarity matrix value (0.65). The affinity of Tifgreen with Bermudagrass-2 indicates that the hybrid is sharing major characters of *Cynodon dactylon* than *C.transvaalensis*. From the low similarity value between Tifgreen and Tifway (0.52) it is assumed that latter is holding major characters donated by its parent, *C. transvaalensis*. Burton (1966) reported that Tifway appeared as a chance hybrid in a seed lot of *Cynodon transvaalensis* Burtt.-Davy from Johannesburg, South Africa, in 1954.

Bermudagrass-1 did not show close similarity with Bermudagrass-2 (0.50). The former may be a selection made by the farmers from the spontaneous mutant originated in the wild forms of *Cynodon dactylon.* This mutant showed more relationship with Tifway (0.64), a hybrid bermudagrass than its wild counterpart. Within species the cultivars showed another tendency of clustering together based on its leaf texture. Miyako and Nagissa, the cultivars of zoysiagrass having coarse texture clustered together while their relative, variant of Miyako with fine texture stood apart. Medium textured cultivars of Bermudagrass like Tifgreen and Bermuda-2 clustered together while the coarse textured Tifway stood apart. Between the species, where there was an anomaly of inter specific clustering of cultivars, the fine textured Bermudagrass-1 formed cluster with fine textured zoysiagrass cultivar.

In general RAPD results of cultivar identification were in agreement with the conventional taxonomic identification using morphologic markers but in some cases discrepancies were found which needs to be explained.

References

Burton, G.W. 1966. Tifway (Tifton 419) Bermudagrass. *Crop Science,* 6: 93-94.

Caetano-Anolles, G. 1998. Review and interpretation: DNA Analysis of turfgrass genetic diversity. *Crop Science,* 38(6): 1415-1424.

Caetano-Anolles, G., B.J. Bassam and P.M. Gresshoff, 1991. DNA amplification fingerprinting: a strategy for genome analysis. *Plant Mol. Biol. Rep*., 9: 294-307.

Chen, Z., W. King, M. Newman, M. Wang and P. Raymer, 2005. Molecular characterization of genetic diversity in the USDA seashore paspalum germplasm collection. *Intl. Turfgrass Soc. Res. J*., 10: 543-549.

Dellaporta, S.L., J. Wood and J.B. Hicks, 1983. A plant DNA minipreperation: Version II. *Plant Mol.Biol. Rep*., 1: 19-21.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from small number of individuals. *Genetics,* 89: 583-590.

Nei, M. and W. Li, 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci.,* USA.76: 5269-5273.

Ohumura, T., T. Sasakuma and S. Kaneko, 1997. Identification of *Poa pratensis* L. and *Agrostis* spp. cultivars by random amplified polymorphic DNA (RAPD) markers. *Intl. Turfgrass soc. Res. J*., 8: 359-365.

Okawara, R., T. Ohumura and N.S. Al-Khalifah, 2002. The response of zoysiagrasses and other grasses to environmental condition of coastal regions of Saudi Arabia. *J. Japan Soc. Turfgrass Sci*., 31 (2): 121-127.

Shabana, H.R. and N.S. Antoun, 1980. Determination of leaf area in date palm. *Hort. Abst*., 51: 9012.

Snedecor, G.W. and W.G. Cochran, 1973. *Statistical Methods* (6th Edn.). Ames, Iowa. USA: Iowa state University Press. pp 305.

Stammers, M., J. Harris, G.M. Evans, M.D. Hayward and J.W. Forster, 1995. Use of Random PCR (RAPD) technology to analyze phylogenetic relationships in the *Lolium/Festuca* complex. *Heredity,* 74: 19-27.

Sweeney, P.M. and T.K. Danneberger, 1994. Random Amplified polymorphic DNA (RAPD) in perennial ryegrass: bulk samples versus individuals. *HortScience,* 29: 624-626.

Sweeney, P.M. and T.K. Danneberger, 1997. RAPD markers from perennial ryegrass DNA extracted from seeds. *HortScience,* 32(7): 1212-1215.

Weaver, K.R., L.M. Callagan, G. Caetano-Anolles and P.M. Gresshoff, 1995. DNA amplification fingerprinting and hybridization analysis of Centipedegrass. *Crop Science,* 35: 881-885.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.K. Rafalski and S.V. Tingey, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res*., 18: 6531-6535.