Evaluation of Genetic diversity of sugarcane variants using morphological characters and RAPD molecular markers.

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ABSTRACT

This study was accomplished to analyze the genetic variation of ٣٥ agricultural sugarcanes variants using RAPD technique. The aim was to determine the genetic variation level and relation among some commercial varieties of *Saccharum officinarum* and the two wild varieties of *Saccharum spontneum* as well. Total genomic DNA of each sample was extracted from ١,٥-٢,٥ gr of new germinated fresh young leaves using modified CTAB method. The concentration was determined by spectrophotometer. RAPD analysis was performed by use of ١٠ bp randomized nucleotide primers. All genotypes were studied for presence or absence of bands, and analyzed by use of statistical software NTSYS ٢٠٠٢. Among ٦٥ used primers, ٣٠

of them showed polymorphism between the sample, and ٤٦٢ polymorphic bands were produced. Cluster analysis lays varieties into two separated groups through the Jacard coefficient and UPGMA methods. In this classification, the two wild varieties from the *Saccharum spontanenm* were separated completely and showed completely different band pattern in comparison with other varieties. Generally, the results of this research showed that RAPD marker is a suitable tool to study genetic variation in sugarcane. It is recommended to increase the efficiency of breeding programs to study precise genetic variation and to determine the relationship among cultivars. Overall, the results indicated that RAPD technique in combination with morphological selection and identification could serve as an ideal tool for studying the genetic variation among sugarcane families.

Key words: Sugarcane, RAPD molecular marker, Genetic variation, morphological characteristics.

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INTRODUCTION

Sugarcane belongs to the grain (*Gramineae)* family and *Saccharum* genus [١, ٢]. In this genus there are three agricultural species of *Saccharum Officinarum*, *Saccharum Barberi* and *Saccharum Sinense* and two wild species of *Saccharum Spontaneum* and *Saccharum Robustum*. Although these species differ in the chromosome number, cross-breeding is possible within the genus [٢].

Sugarcane is considered one of the most important sugar crops worldwide. This crop can produce high quality sugar and in large amount per unit area of land [١, ٣]. Beside sugar, leafs, branches, Bagasse, molasses and chemical products are among sugarcane by-products that have economic value [١]. Because the germplasm modification and breeding is an active process in sugarcane production, study of genetic variation and determination of relative genetic distance between individuals or populations of sugarcane is an important aspect of breeding programs. Furthermore, knowledge in genetic similarities among different genotypes in complement with phenotype information will significantly aid germline modification programs.

آ Considering that genetic analysis of seed sources and seed collections in an organizes and classified manner in an expensive and time-consuming approach, use of inexpensive and simple molecular markers such as Random Amplified Polymorphism DNA (RAPD) could

simplify the process of genotyping and acquiring necessary information regarding the source of crops [٤]. In this method specific sequences within the genome are targeted and amplified using ten nucleotide-long primers in a PCR reaction [٥].

Despite the dominance of the low repeatability of the RAPD technique, this method is used to assess the genetic diversity among plant species and populations [٦]. Leon et al. [٧] used RAPD technique and identified high genetic diversity among ٣٩ species of sugarcane and used the data for breeding programs and selection of suitable parents.آ Nair et al. [٥] used RAPD technique for identification and genetic variation analysis of Indian sugarcanes and recognized specific band patterns for different variants. Karp et al. [٨] used RAPD technique for evaluation of difference in various genotypes and germplasms in *Saccharum* genus and reported poor general variation among the genus. The reason of low genetic diversity among Indian sugarcanes could be explained by repeated used of few parent sugarcanes in the breeding programs [٩].

Considering the importance of sugarcanes in sugar production and agriculture industry of northern and southern of Iran, identification of available genotypes for breeding programs and development of more productive crops is necessary. Identification of productive crops with high yield for cultivation in south regions of Iran enables the breeding program to limit the financial resources to focus on producing crops with the highest yield and modify the genotype for the best quality and quantity product. Identification of high yield crops with similar genotypes and recognizing the genetic diversity among variants results in persistent production and spread of crop with the highest yield in much shorter time.

The purpose of this study is to classify the genotype of the sugarcane crops in Iran and obtain a relationship among different genotypes, and also to study the similarities and differences in the genetic materials of these crops. Such data can be valuable in the breeding programs of the sugarcane crop.

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MATERIALS AND METHODS

Sampling: The genetic material used in this research was consisted of ٣٥ samples from ٢٠٠ domesticated *Saccharum Officinarum* sugarcanes and ٢ samples of wild type *Saccharum Spontaneum* and ٢ *Saccharum hybrid* samples imported to Iran from various sources. This selection was chosen from the earliest and the most recent sugarcanes archived by the **Ahvaz**

Sugarcane Research and Development Center and was selected by the center and included in this study based on yield, variety, adaptation to the region and relationship.

All sample leafs were transferred from Amirkabir Sugarcane Development Center in Ahvaz on ice to Biotechnology Laboratory of Shahrekorde University. After removal of extensions and main veins, leafs were weighted and kept in liquid nitrogen until further application.

DNA extraction: DNA was extracted from ۱,۵-۳ g of leafs using modified CTAB method [۱۰]. DNA was dissolved in Tris-EDTA (TE) buffer and quantified using Eppendorf biophotometer.آ The samples were diluted in a ratio of ۲:۹۸ in TE buffer and read at ۲٦۰ nm. The below formula was used to calculate the amount of DNA in each sample.

A۲٦۰/A۲۸۰ ratio was used to determine the DNA purity. Samples resulted in higher value than ۱,۸ were discarded and DNA extraction was repeated. DNA quality was analyzed using Agarose gel electrophoresis [۱۱].

RAPD technique: Total of ٦۰ primers were synthesis by MWG Biotech Company (Germany) and used for initial evaluation. From which, ۳۰ primers that resulted in constant and sharp bands were selected. The PCR reaction was carried out according to the method described by Williams et al. [۱۲] with minor changes in a ۲۵ آµl reaction. The reaction mixture was consisted of ۲,۵ آµl (۱۰X) PCR buffer, ۲۰۰mM dNTP mixture, ۱۵ ng of each primer, ۰,۷ units of Taq DNA polymerase and ۵۰ ng of template DNA.

After ۳ min of initial denaturation at ۹۳° C, total of ٤٤ cycles of; ۱ min at ۹۲° C, ۱ min at ۳۵° C and ۲ min at ۷۲° C were carried out and followed by final extension of ۱۰ min at ۷۲° C [۱۳].

Presence or lack of each specific band was scored ۱ or ۰ respectively. Data was uniformed using SEQUID analytical software. Weak or un-repeatable bands were ignored. A matrix file was designed, the columns represented genotypes and each rows correspondent to a specific band. A distance matrix and similarity matrix were designed and calculated based on Jaccard similarity coefficient. Classification was carried out by UPGMA method using NTSYS software (۲۰۰۲ Version).

One method to compare the efficiency of different clustering algorithms, is estimation of cophenetic coefficient correlation, goodness of fit criterion for grouping [٤] is the correlation matrix of similarity or distance (Y), by cophenetic matrix (X) by using the options of COPH and MAXCOMP in NTSYS software. The method which shows the highest Cophenetic

coefficient correlation may be considered as the most appropriate method of analysis and could be applicable.

Measurement of quality factors of sugarcanes: The quality factors of each sample were measured by observation in a farm. Scores of ١ to ١٠ were given to quantify the morphological characteristics (Table ١).

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RESULTS

Total of ٦٥ primers were used in this study and ٣٠ of those resulted in repeatable multi-band amplicons with specific bands size (Figures ١). The primers resulted in ٤٨٨ bands with specific sizes. Each specimen showed different band pattern which indicates the genetic diversity among this samples. Band were in a size range of ٢٩٢ bp to ٢٨٢٢ bp. Majority of primers resulted in a distinctive band pattern in wild *Saccharum Spontaneum* compared to all ٣٣ variants of *Saccharum Officinarum*. Including the two wild stains of *Saccharum Spontaneum*; Spont١ and Spont٢, the number of different bands with distinguish patterns were ٤٦٦ which indicated the polymorphism of ٩٣,٩٨٪. The variety of bands in a *Saccharum Officinarum* specie was ٦٦,٤٩٪ and included ٣٣٩ distinct bands (Table ٢).

This result conforms that this technique can be used to classify various genus and species and is a reliable method for using in a breeding programs and genetic reformation of sugarcane species.

Cluster analysis dendrogram divided the samples into two main group with distance of ٧٦٪ (Figure ٢). In this cluster ٣٥ samples were sorted into two mail groups.

First group was divided in ten subgroups which included PINDAR, NI٩, NCo٣١٠ and B٤٢-٢٣١ variants. NI٩ strain was imported from Japan and NCo٣١٠ was imported from Coimbatore, India had a genetic distance of ٢٥٪ and were sorted in one group. Maternal parent of NI٩ strain was NCo٣١٠ [١٤]. Genetic distance of PINDAR variant originated from Australia was ٣١٪ from NI٩ variant. With the exception of B٤٢-٢٣١ variant, all variants of this group had a common ancestor.

The second subgroup comprised from, VESTA and TRITON variants from Australia, TUC٦٨-١٩ variant from Argentina, and C٨٦-٤٥٦ from Cuba. In this cluster VESTA and TRITON

variant had a genetic distance of ۲۹٪ and TUC۶۸-۱۹ and C۸۶-۴۵۶ had a genetic distance of ۳۴٪.

The third subgroup contained the vainest of SP۷۱-۶۱۶۳, SP۷۱-۶۱۱۳ and RB۸۳-۵۵۲۹ from Brazil. The genetic distance of SP۶۱۱۳ and SP۷۱-۶۱۶۳ was calculated ۲۵٪.

The CSG۸۸-۲۶۵ and CSG۸۸-۲۵۸ variants originated from Cuba with the genetic distance of ۲۸٪ were assigned to the fourth group.

The fifth cluster included variants My۵۵-۱۴T C۹۰-۵۳۰ and JA۶۴-۲۰ from Cuba and N۳۰ variant from South Africa. The genetic distance of Ja۶۴-۲۰ and C۹۰-۵۳۰ was ۲۶٪ and the Ja۶۴-۲۰ and My۵۵-۱۴ was calculated ۲۹٪. The genetic distance of N۳۰ variant and Ja۶۴-۲۰ was ۳۱٪.

The sixth subgroup contained ۱۰ strains from Louisiana and Florida, USA and CRISTALINA variant from Guinea. The CRISTALINA variant is a natural hybrid from the *Saccharum Officinarum* genus and is ancestor of these ۱۰ strains.

The seventh subgroup included the two variants of COLK۸۰۰۱ and CO۱۱۴۸ from Coimbatore, India. The COLK۸۰۰۱ is a maternal parent of CO۱۱۴۸.

MEX۵۷-۴۳۷ and COK۳۲ were clustered under the eighth subgroup and SP۷۰-۱۱۴۳ was clustered under the ninth subgroup.

In the second group the wild type *Saccharum Spontaneum* was separated from the other agricultural strains and had a genetic distance of ۵۲٪ (Figure ۲).

The clustering of the obtained data from this research divided the wild-type *Saccharum Spontaneum* from *Saccharum Officinarum* genus. These results are similar to the results obtained by Glaszmann [۱۵] and Schenck [۱۳] who used different molecular markers and is completely in compliance with the RAPD results. Furthermore, our results confirmed the results obtained by Selvi et al [۱۶], Alwala et al [۱۷] and Karami [۱۸] who used SSR and TRAP molecular markers respectively to classify the genus and species of Saccharum in separate dendrogram.

In the morphological classification, the dendrogram plotted based on quality traits divided the samples into four groups (Figure ۳).

The first group is divided into two subgroups. The first subgroup included; CP۷۲-۲۰۸٦, CO۱۱٤۸, CRISTALINA, CL۷۳-۲۳۹ and B٤۲-۲۳۱. The second subgroup included three variants of Spont۱, Spont۲ and MEX٥۷-٤۷۳. All species in the first groups have raised leafs. In contrast to the second subgroup, the first subgroup had less adherent sheaths to the stem. Leafs in the first subgroup were all green or light green and the second subgroup variants had dark-green leafs. Unlike the second group, in the first group all stem had waxy texture. All members of group one had a cylindrical internodes. None of the second group members had corky leave, whilst, CRISTALINA, CP۷۲-۲۰۸٦ and CP٤۸-۱۰۳ variant from the first group had corky leave.

The first group is separated from the second group with a distance of ٤,۱۰. The second group is divided into tree subgroups, the first and the second of those are separated at the distance of ۲,۰۷, and the second subgroup is separated from the third subgroup at the distance of ۳,۰۸.

The second group comprised from; NCO۳۱۰, NI۹ TRITON, CP۷۰-۳۲۱, CP۷۳-۲۱, L٦۲-٦۹, N۳۰, JA٦٤-۲۰, COLK۸۰۰۱, RB۸۳-٥٥۲۹. Members of this group have raised and semi-raised leafs and the adherent of the sheath to the stem is intermediate to strong. Leafs are dominantly green, except for NCO۳۱۰, CP۷۳-۲۱, and CP۷۰-۳۲۱ who had brown lines within a green background. Most stem contained cylindrical internodes. In the second subgroup of the second group, only the NI۹, TRITON, and L٦۲-٦۹ variants had corky leaves. The leaf color is dominantly green, however some variants had green to yellow features. The second group is divided from the tired one at the distance of ٤.٦۷.

The third group comprised from CSG۸۸-۳٥۸, VESTA, SP۷۰-۱۱٤۳, CP٥۷-٦۱٤ and TUC٦۸-۱۹. Leafs of the third group are semi-raised or flat. The sheaths have strong adherence to the stem. Leafs are usually dark-green and in the two variants of SP۷۰-۱۱٤۳ and CP٥۷-٦۱٤ stems have high waxy texture whereas in the TUC٦۸-۱۹, CSG۸۸-۳٥۸ and VESTA the stems have less waxy texture. The internodes are cylindrical and in the CSG۸۸-۳٥۸, SP۷۰-۱۱٤۳ and CP٥۷-٦۱٤ the internodes are narrower. The members of this group lack corky leaves and stem are dominantly green. The fourth group is separated from the third group at the distance of ٥,٦۲. The fourth group includes three subgroups, and the first subgroup has distance of ۲,۷۹ from the second subgroup which itself is separated from the third subgroup at distance of ۳,٥٤.

The CP٦۳-٥۸۸, CSG۸۸-۳٦٥ ,PINDAR, MY٥٥-۱٤, SP۷۱-٦۱۱۳, SP۷۱-٦۱٦۳, CP٦۲-۱۰٦۲ and CP٥٦-٦۹ variant are classified into the fourth group. These variants have raised leafs. The

adherence of sheath is weak to intermediate. Leafs are dark-green and three variants of SP٧١-٦١٦٣, PINDAR and SP٧١-٦١١٣ have opaque dark-green leafs. In this group only MY٥٥-١٤ has light-green leafs. The stem of this variant has less waxy texture. In CP٦٣-٥٨٨ and MY٥٥-١٤ the internodes are barreled-shaped which is unique among all members. CP٦٣-٥٨٨ and MY٥٥-١٤ have corky leave. In all members except CP٦٣-٥٨٨ the stem has red color.

Generally in the dendrogram plotted based on the quality of the traits, the two variants of TRITON and L٦٢-٦٩ had highest similarly and two variants of SP٧٠-١١٤٣ and CP٥٧-٦١٤ had the lowest similarity rate.آ

The highest correlation between the quality traits belonged to the color of the leaves and stems texture and the lowest correlation belonged to the waxiness of the stem and the shape of leafs.

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DISCUSSION

The RAPD technique used in this research was able to completely distinct between the sugarcane genus and even within the different species used in the study the obtained band pattern was unique and distinguishable.

These results are consistent with the reports published by other researchers including Huckett et al [١٩] and Barأ؛nek et al [٢٠] who reported RAPD bands are inherited in sugarcane families and can be used as a molecular marker to distinguish different variants of the crops.

The cophenetic coefficient correlation in this study was estimated to be R= ٠,٠٩٨ which indicates a good correlation between the dendrogram and similarity matrix of varieties [٢١]. The cophenetic coefficient correlations of Râ‰¥ ٠,٩, ٠,٨â‰¤Râ‰¤٠,٩, ٠,٧â‰¤Râ‰¤٠,٨ and R< ٠,٧ shows correlation of very good, good, week, very week respectively. However, the low cophenetic coefficient correlation in a molecular study does not inefficiency of the algorithm, rather is shows difference in the data due to misplacement of some information [٢٢].

In a closer look at the obtained dendrogram we realize that in addition to the different clustering of agricultural and wild-type strains, the samples were also divided based on parents, origin and similar nomenclature. However, among this ٣٥ variant, some heterogeneous variants that had similar names or origin were included in different clusters. Schenck et al [١٣] in a study on domesticated and agricultural sugarcane variant which had

similar naming and was expected to have identical clustering, reported that variants were divided into different clustering groups. Additionally, the new developed spices have high polyploidy and it is expected to have significant chromosomal overlap, thus there are more annealing sites for RAPD primers. The classification of these species using RAPD will not be useful and result in various and unrepeatable bands, however, this technique can be useful for diversity studies.

The results of cluster analysis based on the traits quality did not provide any useful data regarding the functionality of the traits and only revealed the correlation among the quality traits.

In this research there was a suitable diversity in the statistics and with the use of genetic distance calculated for each group, proper parents can be identified for breeding programs.آ Since the strains with the highest genetic distance have higher heterosis potential, cross between parents with the highest genetic distance will have better yield in production of hybrid strains

The overall results of this study shows that RAPD technique is a proper tool for analysis of genetic diversity in sugarcanes and it is recommended that for better yields in the breeding program, the strains with the highest genetic distance to be used. Example of that will be the cress of NCO۳۱۰ strain with the MEX۵۷-٤۷۳ strain. If the selection of breeding parents in the breeding programs would be solely based of morphological characteristics, the results may not be desirable.

Before use of molecular markers for analysis genetic relationships in certain species, morphological characteristics had the most significant role. Recently biotechnological tools has significantly improved phylogenic studies and detection of relationship among families of certain genus. Use of molecular markers and morphological traits are complementary in the breeding programs and separate use of each method without considering the other one may not lead to the best results. There are various studies that used statistical analysis and reported correlation between a molecular marker and a specific trait in plants [۲۳-۲٦].

Our results indicated that morphological characteristics are useful in identification of genetic diversity in sugarcanes. However, RAPD technique uses molecular markers and signatures and was successful in identification of genetic diversity. The RAPD technique is a useful tool for obtaining genetic diversity among different species and even within a family of sugarcanes and can be used to manage the germplasm resources.

The clustering results based on RAPD technique and morphological traits show somewhat similarities. The differences could be explained by post translational modification and non-nuclear inheritance of some traits. Therefore, as shown in this study, the diversity of genetic material in different species such as sugarcanes can be measured by a simple and cost-effective molecular method such as RAPD and the results can be used with high accuracy.

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REFERENCES

١. Barat Shooshtari, M., Ahmadian, S. and Asfya, G. Sugarcane in Iran. ٢٠٠٨. ١

٢. Yazdi Samadi, B., Abd Mishani, S. and Rahi, M. Breeding crop. ٢٠٠٧. ٦

٣. Khajepour, M. Production of industrial crops. ١٩٩٨. ٤

٤. Katsiotis, A., Hagidimitriou, M., Drossou, A., Pontikis, C. and Loukas, M. Genetic relationships among species and cultivars of Pistacia using RAPDs and AFLPs. *Euphytica.* ٢٠٠٣آآ vol. ١٣٢ (٣). pp. ٢٧٩-٢٨٦.

٥. Nair, N., Selvi, A., Sreenivasan, T. and Pushpalatha, K. Molecular diversity in Indian sugarcane cultivars as revealed by randomly amplified DNA polymorphisms. *Euphytica.* ٢٠٠٢آآ vol. ١٢٧ (٢). pp. ٢١٩-٢٢٥.

٦. Greene, S., Gritsenko, M. and Vandemark, G. Relating Morphologic and RAPD marker varlation to collection site environment in wild populations of Red Clover (Trifolium pratense L.). *Genetic resources and crop evolution.* ٢٠٠٤آآ vol. ٥١ (٦). pp. ٦٤٣-٦٥٣.

٧. Leon, F., Prodriguez, J., Azurdia, C., Amador, D., Queme, J. and Melgar, M. Use of RAPD markers to detect genetic variability among ٣٩ sugarcane varieties. *Proc. Int. Soc. Sugar Cane Technol*. ٢٠٠١. ٢٤ ٦٣٤-٦٣٦.

٨. Karp, A., Seberg, O. and Buiatti, M. Molecular techniques in the assessment of botanical diversity. *Annals of Botany.* ١٩٩٦آآ vol. ٧٨ (٢). pp. ١٤٣-١٤٩.

٩. Roy, D. Plant Breeding: Analysis and exploitation of variation. ٢٠٠٠.

١٠. Murray, M. and Thompson, W. F. Rapid isolation of high molecular weight plant DNA. *Nucleic acids research.* ١٩٨٠آ vol. ٨ (١٩). pp. ٤٣٢١-٤٣٢٦.

١١. Popi, J., Rajnpreht, J., Kannenberg, L. and Pauls, K. Random Amplified Polymorphic DNAâ€"Based Evaluation of Diversity in the Hierarchical, Open-Ended Population Enrichment Maize Breeding System. *Crop science.* ٢٠٠٠آ vol. ٤٠ (٣). pp. ٦١٩-٦٢٥.

١٢. Williams, J. G., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingey, S. V. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic acids research.* ١٩٩٠آ vol. ١٨ (٢٢). pp. ٦٥٣١-٦٥٣٥.

١٣. Schenck, S., Crepeau, M., Wu, K., Moore, P., Yu, Q. and Ming, R. Genetic diversity and relationships in native Hawaiian Saccharum officinarum sugarcane. *Journal of heredity.* ٢٠٠٤آ vol. ٩٥ (٤). pp. ٣٢٧-٣٣١.

١٤. Pí©rez, G., Bernal, N., Chinea, A., Oâ€™relly, J. and Prada, F. D. Recursos gení©ticos de la caí±a de azúcar. *Instituto Nacional de Investigaciones de la Caí±a de Azúcar. Ciudad de La Habana, Cuba.* ١٩٩٧آ vol. pp.

١٥. Glaszmann, J., Fautret, A., Noyer, J., Feldmann, P. and Lanaud, C. Biochemical genetic markers in sugarcane. *Theoretical and applied genetics.* ١٩٨٩آ vol. ٧٨ (٤). pp. ٥٣٧-٥٤٣.

١٦. Selvi, A., Nair, N., Balasundaram, N. and Mohapatra, T. Evaluation of maize microsatellite markers for genetic diversity analysis and fingerprinting in sugarcane. *Genome.* ٢٠٠٣آ vol. ٤٦ (٣). pp. ٣٩٤-٤٠٣.

١٧. Alwala, S., Suman, A., Arro, J. A., Veremis, J. C. and Kimbeng, C. A. Target region amplification polymorphism (TRAP) for assessing genetic diversity in sugarcane germplasm collections. *Crop science.* ٢٠٠٦آ vol. ٤٦ (١). pp. ٤٤٨-٤٥٥.

١٨. Karami, S. Analysis of the sugarcane genetic varianys using morphological traits and RAPD molecular marker. . *Faculty of Agriculture.* ٢٠٠٦. Master of Science ١٠٥.

١٩. Huckett, B. and Botha, F. Stability and potential use of RAPD markers in a sugarcane genealogy. *Euphytica.* ١٩٩٥آ vol. ٨٦ (٢). pp. ١١٧-١٢٥.

٢٠. Bar،أnek, M., Raddov،أ, J. and Pidra, M. Comparative analysis of genetic diversity in *Prunus* L. as revealed by RAPD and SSR markers. *Scientia horticulturae.* ٢٠٠٦آ آ vol. ١٠٨ (٣). pp. ٢٥٣-٢٥٩.

٢١. Rohlf, F. NTSYS-pc version ٢،٠. *Numerical taxonomy and multivariate analysis system. Exeter software, Setauket, New York.* ١٩٩٨آ آ vol. pp.

٢٢. Mohammadi, S. and Prasanna, B. Analysis of genetic diversity in crop plantsâ€"salient statistical tools and considerations. *Crop science.* ٢٠٠٣آ آ vol. ٤٣ (٤). pp. ١٢٣٥-١٢٤٨.

٢٣. Martأnez, L., Cavagnaro, P., Masuelli, R. and Rodrأguez, J. Evaluation of diversity among Argentine grapevine (Vitis vinifera L.) varieties using morphological data and AFLP markers. *Electronic Journal of Biotechnology.* ٢٠٠٣آ آ vol. ٦ (٣). pp. ٢٤٤-٢٥٣.

٢٤. Mcharo, M., Labonte, D., Oard, J., Kays, S. and Mclaurin, W. Linking quantitative traits with AFLP markers in a sweetpotatoes using discriminant analysis. *Acta horticulturae.* ٢٠٠٤آ آ vol. pp.

٢٥. Mcharo, T. M. Associating molecular markers with phenotypes in sweetpotatoes and liriopogons using multivariate statistical modeling. ٢٠٠٥.

٢٦. Rold،أn-Ruiz, I., Van Euwijk, F., Gilliland, T., Dubreuil, P., Dillmann, C., Lallemand, J., De Loose, M. and Baril, C. A comparative study of molecular and morphological methods of describing relationships between perennial ryegrass (Lolium perenne L.) varieties. *Theoretical and applied genetics.* ٢٠٠١آ آ vol. ١٠٣ (٨). pp. ١١٣٨-١١٥٠.

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Table ١; Traits analyzed in morphological study.

	of the Stem Color	**corky of leave**	**Shape of the internodes**	**Waxiness of stem**	**Color of the the leaf**	**Adherence of sheath**	**Shape the leaf**
Yellow-Green Yellow-red	Yellow	semi- Yeas	cylindrical cone	low	Green	low	Raised
Green-yellow Green-Red	Green		Narrowed Barreled-shape	Intermediate	Light- Green	Intermediate	Semi- raised
Light-red Dark-red Combination of yellow, green and red	Red	No	Curved	high	Dark- Green	high	Flat

Table ۲: Results of RAPD. Number of bands, polymorphism and the name of primer used are indicated

Name of Primer	Total of bands	Number of polymorphic bands	Percentage of polymorphic bands
OPM۲	٦	٤	٦٦,٦٦
OPM٤	١٨	١٨	١٠٠
OPM٥	٢٢	٢٢	١٠٠
OPM٨	١٩	١٩	١٠٠
OPM١١	١١	٨	٧٢,٧٢
OPM١٥	٩	٩	١٠٠
OPM١٦	١٦	١٥	٩٣,٧٥
OPM١٧	١٨	١٨	١٠٠
OPM١٨	١٢	١١	٩١,٦٦
OPM١٩	١١	١٠	٩٠,٩٠
OPM٢٠	١٨	١٨	١٠٠
OPM٢١	٢١	٢١	١٠٠
OPM٢٢	١٨	١٧	٩٤,٤٤
OPM٢٣	١٦	١٥	٩٣,٧٥
OPM٢٥	١٧	١٥	٨٨,٢٣
OPM٢٧	١١	١١	١٠٠

OPM۲۹	۱۵	۱۵	۱۰۰
OPM۳۲	۱۵	۱۳	۸۶,۶۶
OPM۳۳	۲۷	۲۲	۱۰۰
OPM۳۵	۲۲	۲۲	۱۰۰
OPM۳۶	۱۶	۱۶	۱۰۰
OPM۳۷	۱۶	۱۶	۱۰۰
OPM۳۸	۱۷	۱۵	۸۲,۲۳
OPM۴۰	۱۳	۱۱	۸۴,۶۱
OPM۴۲	۱۷	۱۷	۱۰۰
OPM۴۸	۲۰	۲۰	۱۰۰
OPM۵۰	۲۲	۲۲	۱۰۰
OPM۵۵	۲۱	۲۱	۱۰۰
OPM۵۷	۱۰	۶	۶۰
OPM۶۰	۱۴	۱۰	۹۳,۳۳

Figure ۱: Electrophoresis results of amplified DNA using random primers,

From top to down, respectively; OPM(۵۵).

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Figure ۲: Plotted dendrogram based on Jaccard coefficient for ۳۵ variants of sugarcane based on RAPD results.

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Figure ۳: Classification of sugarcanes based on quality of traits using Jaccard coefficient.