RAPD and ISSR analyses of *Saccharomyces cerevisiae* isolates from different sources

تحليل RAPD وISSR ل *Saccharomyces cerevisiae* المعزولة من مصادر مختلفة

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Abstract

The purpose of this study was to isolate the *Saccharomyces cerevisiae* present on different fruits and performing RAPD and ISSR analyses to know the genetic interrelationship between different *S. cerevisiae* isolates. Some fruits namely apple, plum, dates, and peach were used as natural sources for *S. cerevisiae* isolation. The isolated *S. cerevisiae* was designated as SUC1, SUC2, SUC3, SUC4, SUC5 respectively. Amplicon fingerprints for the isolated species were obtained by RAPD assay using six different primers and ISSR assay using six different primers. RAPD assay showed the lowest genetic distance (0.1559) between SUC2 and SUC3 isolates whereas ISSR assay showed the lowest genetic distance (0.06899) between SUC4 and SUC5 isolates. Both genetic markers showed the highest genetic distance for SUC1 when compared to the other isolates.

Key words: RAPD and ISSR assay, *Saccharomyces cerevisiae*, Bread yeast, Apple, Plum, Dates, Peach.

الخلاصة:

الغرض من هذه الدراسة هو استخدام التحليل الجزيئي RAPD وISSR لعزل الخمائر *Saccharomyces cerevisiae* الموجودة على ثمار مختلفة تمامًا وتحليل RAPD وISSR من أجل فهم العلاقة الجينية بين العزلات المختلفة. استخدمت بعض الفواكه وعلى وجه التحديد التفاح والبرقوق والتمر والخوخ كمصادر طبيعية لعزل *S. cerevisiae* بالإضافة الى خميرة الخبز. تم الحصول على بصمات وراثية للأنواع المعزولة بواسطة اختبار RAPD باستخدام ستة بادئات مختلفة تمامًا واجراء تحليل ISSR باستخدام ثمانية أنواع مختلفة من البادئات. تحليل ال RAPD اظهر ان العزلات المأخوذة من خميرة الخبز والتفاح لها اقل بعد وراثي 0.1559. تحليل ال ISSR اظهر ان العزلات المأخوذة من البرقوق والخوخ لها اقل بعد وراثي 0.06899. كلا المؤشرين اظهر ان العزلات المأخوذة من التمر لها اعلى بعد وراثي مع العزلات الأخرى.

الكلمات المفتاحية: تحليل ISSR و RAPD , *Saccharomyces cerevisiae* , خميرة الخبز، تفاح، برقوق، تمر، خوخ.

Introduction

Saccharomyces cerevisiae is a unicellular eukaryotic fungus, very common in the environment and is mostly saprophytic because their cells do not contain chlorophyll. [1]. *S. cerevisiae* comes in the forefront of microbiological groups used in the industrial and production areas as well as most consumed by humans where they are safe and produce non-toxic materials [2], It has been made as a model organism of study on both research and industrial importance [3]. Fermenting wild yeast species are being isolated from the natural sources for over decades and is being used in various fermentation processes. *S. cerevisiae* feeding is of the heterotrophic type so it has been isolated from a variety of natural sources like leaves, flowers, fruits etc. [4, 5]. Being a sugar-loving microorganism, it is usually isolated from sugar-rich materials. Fruits contain high sugar concentration so yeast species are naturally present on them and can be easily isolated from them. Distinct wild yeast species are supposed to be present and associated with different fruits in natural environments [6]. Traditional methods like morphological, physiological and biochemical studies used for taxonomic identification of yeast isolates [7, 8]. But being laborious and time-consuming techniques, they are not appropriate for routine identification [9]. Molecular biology-based methods have been developed and can be applied to the field of yeast taxonomy in routine identification works [10]. One of the diagnostic methods for *S. cerevisiae* is Polymerase Chain Reaction (PCR), which can amplify some specific fragment of DNA into millions of copies [11, 12]. In recent years, different molecular typing techniques have been applied to study the genetic diversity of *S. cerevisiae* and the possible

occurrence of similarity and difference between them, Random Amplified Polymorphic DNA (RAPD) analysis can be performed as a method for study genetic diversity with a large number of different strains of microorganisms. It is inexpensive and requires less amount of DNA [13]. Moreover, RAPD analysis is technically being commonly used as an indicator for determining the genetic diversity, while Inter-simple sequence repeat technique ISSR analysis based on variation found in the regions between microsatellites it has been used in genetic fingerprinting gene tagging and detection of clonal variation [14]. This technique which involved amplification of DNA segment present in between two identical microsatellite repeat regions by addition the oriental in opposite direction with suitable distances ISSR method has been reported produce more complex markers patterns than the RAPD markers in addition, ISSR method are more reproducible than RAPD method because ISSR primers designed to anneal temperature to a microsatellite sequences are long than RAPD primers, allowing higher annealing temperature to be used. It also because of multilocus fingerprinting profile obtained ISSR has been found to be an efficient, low cost, simple operation, high stability and abundance of [15, 16]. The aim of the study was to determine the effect of the nutritional components on the genetic components of the yeast as well as to detect the unique bands and polymorphism between isolates and to compare between RAPD and ISSR markers for genetic diversity between different *S. cerevisiae* isolates.

Materials and Methods

Isolation of *S. cerevisiae*

A total of five ***S. cerevisiae*** and fruit source isolates as shown in (Table-1)

Table (1): *Saccharomyces cerevisiae* isolates examined during this study

***S. cerevisiae* isolates**	***S. cerevisiae* fruit source**	
	Common name	Scientific name
***SCU*1**	Dates	***Phoenix dactylifera***
***SCU*2**	Bread yeast	***Saccharomyces cerevisiae***
***SCU*3**	Apple	***Malus pumila***
***SCU*4**	Plum	***Prunus cultivar***
***SCU*5**	Peach	***Prunus persica***

Fruit samples were obtained from local markets, 100 g of each fruit sample was taken in a sterile mortar and crushed to a fine paste by mixing with sterile water. Then, the mixture was kept for overnight at room temperature so that natural wild yeast present on fruit samples might grow and develop to enrichment culture. As for bread yeast, 0.1 g of dry yeast was suspended in tubes containing 10 mL. of sterile distilled water and incubated for 30 min at 30 ° C and the stuck as with Vortex. Streaked an equal amount of the liquid portion of each sample and planted on the Yeast Extract Peptone Dextrose (YEPD) medium (This medium is prepared by dissolving 20 g glucose, 20 g peptone and 10 g yeast extract in 1 litre of distilled water and adding 20 g of agar with pH 5 and then sterilized by autoclave. This medium is used to stimulate, diagnose and develop *S. cerevisiae* isolates [17]. Streaked plates were incubated at 30± ° C for 48 h. After the emergence of growth taking part in a single colony was streaked onto the anew YPED plate and incubated at 30±°C for 48h. This process was repeated until a pure culture was obtained which was confirmed by examining the cells under a light microscope.

Genomic DNA extraction

The DNA was extracted by small-scale methodology industrial kit (Bioneer-Korea). DNA purity was measured counting on optical density by spectrophotometer. DNA quality was determined by agarose gel electrophoresis stained 0.5 mg/ml with ethidium bromide and visualized under Ultra Violet light [18].

Molecular Analysis

RAPD assay

Six of RAPD primers were utilized in this study, the primers were synthesized by (Bioneer-Korea) in lyophilized type and dissolved in sterile water to obtain a final concentration of (10pmol/ml), [19, 20, 21]. The primers and their sequences are listed in Table(2).

Table (2): The names and sequences of the primers used in this study

No.	Primers name	Sequences (´5 -´3)
1	OPI – 06	AAGGCGGCAG
2	OPE-16	GGTGACTGTT
3	OPN-07	GAGCCCCGAG
4	OPQ-17	GAAGCCCTTG
5	OPD-20	ACCCGGTCAC
6	OPL-05	ACGCAGGCAC

Amplification of genomic DNA was performed with the master amplification reaction as illustrated in Table (3).

Table (3): Master reaction of RAPD

Materials	Volume for 1 tube µl	Final concentration
PCR premix	5	1x
Deionised D.W	11	——
Primer (10 pmol/µl)	2	10 pmol /µl
DNA template	2	100 ng

RAPD – PCR premix (final reaction volume = 20 µI). No. of cycles = 40 cycles between initial denaturation and final extension, was shown in Table (4).

Table (4): The RAPD assay program

Steps	Temperature (°C)	Time (min)	Number of cycles
Initial denaturation	94	5	1
Denaturation	94	1	
Annealing	36	1	35-40
Extension	72	2	
Final extension	72	10	1

Each PCR amplification reaction was continual double to confirm dependability the products analyzed by electrophoresis in 1-1.5% agarose with 0.5µl stained ethidium bromide at 5v/cm for 2h.

ISSR assay

Six of ISSR primers were used they were provided by (Bioneer – Korea) in lyophilized type and dissolved in sterile distilled water to obtain a final concentration of (10 pmol/ml) [19, 22]. as recommended by the supplier. The primers that tested during this study are listed in Table (5).

Table (5): The names and sequences of the primers used in this study

No	Primers Name	Sequence (´5-´3)
1	$ISSR_2$	GACAGACAGACAGACAGACAA
2	$ISSR_6$	AGAGAGAGAGAGAGAGAGAGC
3	$ISSR_7$	AGAGAGAGAGAGAGAGAGAGT
4	$ISSR_8$	CTCTCTCTCTCTCTCTCTCTA
5	$ISSR_9$	CTCTCTCTCTCTCTCTCTCTG
6	$ISSR_{10}$	CTCTCTCTCTCTCTCTCTCTT

Following master amplification reaction as represented in Table (6).

Table (6): Master reaction of ISSR

Materials	Volume for 1 reaction (µl)	Final concentration
PCR premix	5	1x
Deionised D.W	17	——
Primer (10 pmol/µl)	2	10 pmol /µl
DNA template	1	100 ng

The final concentration was performed in a volume of 20-25µl. PCR program for ISSR assay exploitation the subsequent program which showed in Table (7).

Table (7): The ISSR assay program

Steps	Temperature (°C)	Time (min)	Number of cycles
Initial denaturation	94	5	1
Denaturation	94	1	
Annealing	50	1	35-40
Extension	72	1	
Final extension	72	10	1

Each PCR amplification reaction was repeated twice to ensure reproducibility the products analyzed by electrophoresis in 1-1.5% agarose with stained ethidium bromide 0.5µl at 5v/cm for 2h.

Data analysis

Estimation of molecular weight

Computer software Photo-Capture M.W. program was used to determine molecular weight based on comparing the RAPD-PCR and ISSR-PCR products with molecular weight marker bands from (Bioneer – Korea) (which consist of 13 bands from 100 to 2000 bp.)

Estimation of polymorphism, efficiency and discriminatory power .

Data generated for molecular weight RAPD and ISSR markers result bands were scored for each band on the molecular size (1 for a present, 0 for absence) the commercial software [23, 20, 24, 25]. Only major bands consistently amplified were scored. Polymorphism of each primer was calculated based on the following formula:

$$\text{Polymorphism \%} = (Np / Nt) \times 100$$

Where Np = the number of polymorphic bands of random primer, Nt = the total number of bands of the same primer. Efficiency and discriminatory power of each primer calculated according to the formula below:

• Efficiency =number of the polymorphic bands to each primer / total number of bands to the same primer.

• Discriminating power= number of the polymorphic band to each primer / total number of polymorphic bands to all primer ×100 %.

Primer efficiency ranged between (0-1). Discrimination power of each primer.

Results and Discussion

5 yeast species were isolated, purified and morphological as well as genetic diversity analysis using RAPD and ISSR methods were successfully carried out. Yeast specific defined media used was able to inhibit the growth of bacterial population in all the cultures. Some fungal contamination was seen after 3 days of incubation of primary cultures. Pure cultures were easily made by taking part of the colonies by a loop and streaked on the medium and repeated more than once that the colonies were obtained individually and pure yeast culture became dominant (that growth after 7 days). Morphological data based on different parameters of colony characteristics are tabulated in Table (8).

Table (8): colony morphological data of different *S. cerevisiae* isolates

S. cerevisiae isolates	**Color**	**Shape**	**Surface**	**Elevation**	**Edge**
SCU1	Cream	Oval	Glistening	Flat	Curled
SCU2	White	Circular	Smooth	Raised	Curled
SCU3	White	Circular	Wrinkled	Bulged	Curled
SCU4	White	Circular	Smooth	Convex	Curled
SCU5	White	Circular	Wrinkled	Convex	Curled

The five isolates, in general, showed common morphological characteristics. Common characteristics were found between SCU3 with **SCU5** compared to the other species. A similarity was also observed between the **SCU4** and **SCU5**. While **SCU1** showed more variation than other isolates. Dates contain 75% sugars compared to other isolates, the presence of high concentration of sugar in the food medium leads to the formation of large amounts of alcohol even under the conditions of air, as the yeast is negatively affected by alcohol produced by the food medium [26, 27]. These results are similar to a study [28, 29, 30].

RAPD-PCR analysis

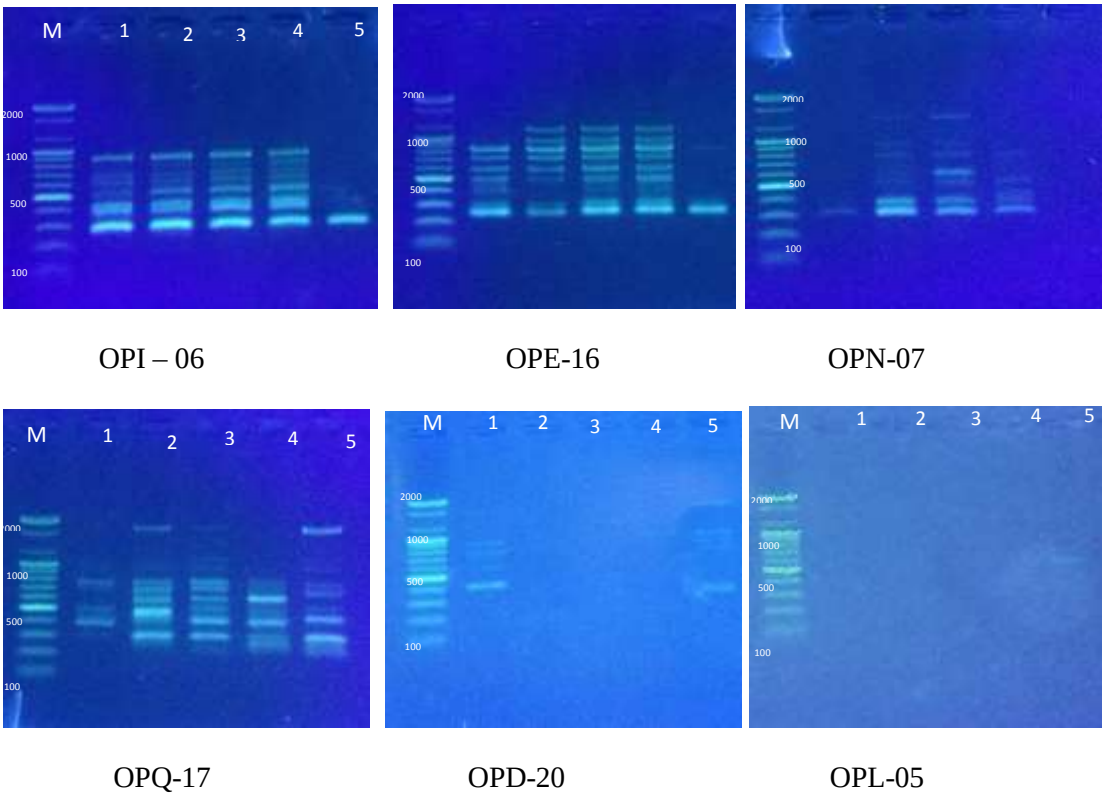

Figure (1): PCR amplifications RAPD primer on 1-1.5% agarose gel electrophoresis with ethidium bromide, M=1000 bp. Lines= *S. cerevisiae* isolates (SCU1, SCU2, SCU3, SCU4, SCU5), (1X TBE, 5v/cm, 2h, 0.5 mg/ml ethidium bromide)

Based on RAPD assay the data developed from the PCR analysis demonstrated that some primers generate several bands, while other generates only a few bands. A total of six RAPD primers were used for study the genetic differences between five *S. cerevisiae* isolates, amplified 111 bands, 101 bands were polymorphic, with average of (1-26) polymorphic bands, that OPD-20 produce 1 polymorphic band only, were OPN-17 can be produce 26 polymorphic bands with average range size (300-1700) bp. Some isolates could be distinguished from all other isolates with a selection of these primers, for instance, OPI-06 primers can produce lower discrimination power 80.7 bands only, while OPL-05 gave 4 unique bands patterns Table (5).

Table (9): Distinct characteristics of RAPD primers included in the study: primers name, total number of bands, number of polymorphic bands, number of unique bands, percentage of polymorphism, primer efficiency and discrimination value.

No.	Name of primers	Total number of main bands	Number unique bands	Number polymorphic bands	Polymorphism ٪	Primer efficiency	Discrimination power
1	P1	26	1	21	3.84	0.8	80.7
2	P2	19	2	19	5.26	1	100
3	P3	29	1	24	3.44	0.82	82.7
4	P4	26	0	26	3.84	1	100
5	P5	1	1	1	100	1	100
6	P6	10	4	10	10	1	100

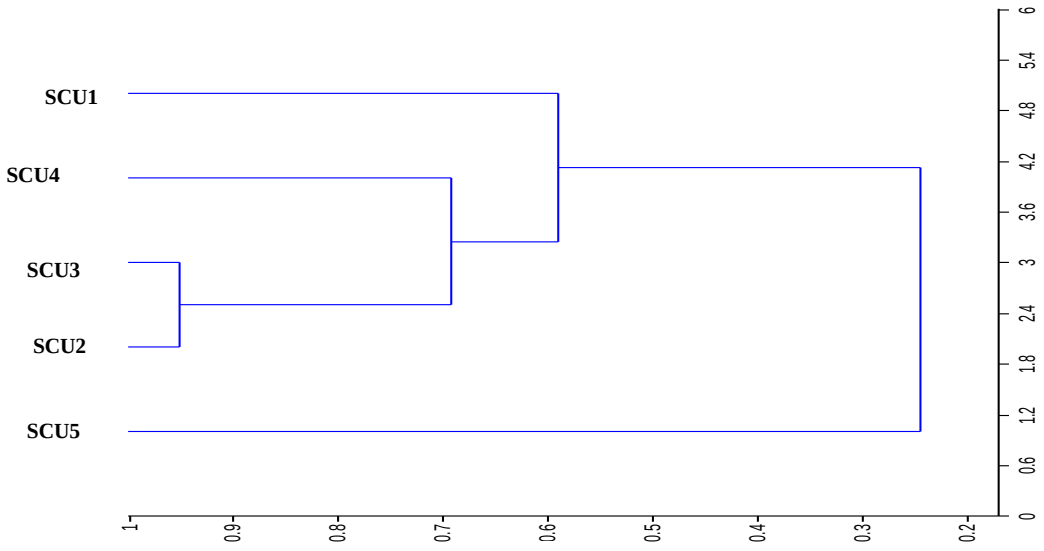

Figure (2): Dendrogram illustrated genetic fingerprint and relationship between *S. cerevisiae* isolates developed from RAPD data.

Table (10): Values of genetic distance among *S. cerevisiae* Isolates calculated according to Nei and Lei, 1979).

No.	1	2	3	4	5
1	0.0000				
2	0.89781	0.0000			
3	0.78148	0.1559	0.0000		
4	0.75588	0.8049	0.69503	0.0000	
5	1.3497	1.1466	0.98821	1.3105	0.0000

The dendrogram clearly showing the sufficient distance to form apart clusters. Cluster analysis illustrated a genetic relationship among five of ***S. cerevisiae*** isolates showing two major clusters (figure-2), the first cluster contained **SCU2** and **SCU3** isolates with low genetic distance 0.1559. These were introduced

from **bread yeast** and **apple** sources respectively. This indicates the genetic similarity between the isolated species of the yeast and the apple and this was not apparent in the form of the colonies where the colony was taken from the yeast of bread more like the colony taken from this plum illustrates the accuracy of genetic analysis compared to the analysis of the phenomenon. Isolate **SCU1** and **SCU4** formed a separated line, while the second group contained **SCU5**. Table (8) illustrates the highest genetic dimension between the sample taken from dates and the sample taken from peach.

ISSR-PCR analysis

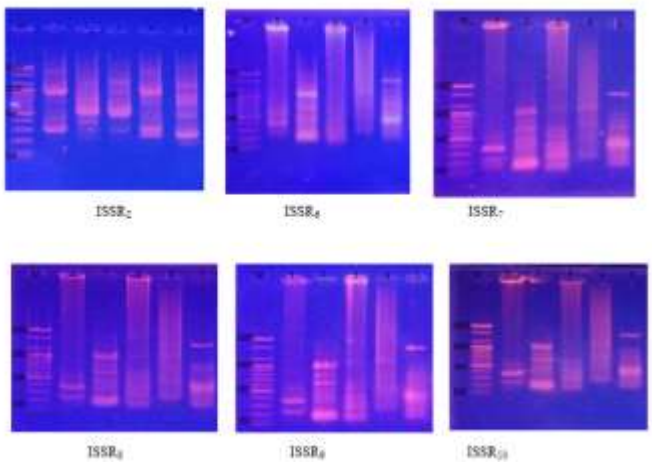

Figure (3): PCR produced ISSR primer on 1-1.5% agarose gel electrophoresis with ethidium bromide, M=1000 bp. , Lines= *S. cerevisiae* isolates (SCU1, SCU2, SCU3, SCU4, SCU5), (1X TBE, 5v/cm, 3h, 0.5mg/ml ethidium bromide).

In this study, ISSR-PCR technique was used to reveal the genetic diversity among different studied *S. cerevisiae* isolates in order to search the genetic diversity between *S. cerevisiae* isolates and study the differences that come from the environment. A total of 145 using full bands were scored from the amplified products with the eight Inter-Simple Sequence Repeat (ISSR), 100 bands were polymorphic, with the average of 4 polymorphic bands ISSR4, and ISSR3, ISSR7 produce 19 polymorphic bands with average range size (250-1600) bp. (figure-3). ISSR8 primers can produce high unique bands can produce 5 unique bands, Table(7).

Table (11): Distinct characteristics of ISSR primers including in the study: primers name, total number of bands, number of polymorphic bands, number of unique bands, percentage of polymorphism, primer efficiency and discrimination value.

No.	Name of primers	Total number of main bands	Number of unique bands	Number of polymorphic bands	Polymorphism %	Primer efficiency	Discrimination power
1	P1	15	2	10	6.66	0.66	66.6
2	P2	16	0	6	6.25	0.37	37.5
3	P3	19	0	19	5.26	1	100
4	P4	9	0	4	11.1	0.44	44.4
5	P5	19	1	9	5.26	0.47	47.3
6	P6	22	1	17	4.54	0.77	77.2
7	P7	24	3	19	4.16	0.79	79
8	P8	21	5	16	4.76	0.76	76.1

From the genetic distance, the ratio of genetic similarity among the five *S. cerevisiae* isolates from 0.65791 to 0.06899 (table-9). The highest genetic distance 0.65791 between isolate SCU1, SCU3 and SCU4 respectively. While 0.41926 genetic distance between isolates SCU4 and SCU5 with isolate SCU2, but the

lowest level of genetic distance 0.06899 appeared between isolate SCU4 and SCU5 which Indicates the genetic similarity because they belong to the same genus Prunus.

Table (12): Values of genetic distance among *S. cerevisiae* Isolates calculated according to Nei and Lei, 1979).

No	1	2	3	4	5
1	0.00000				
2	0.51285	0.00000			
3	0.65791	0.37274	0.00000		
4	0.65791	0.41926	0.51083	0.00000	
5	0.58380	0.41926	0.40547	0.06899	0.00000

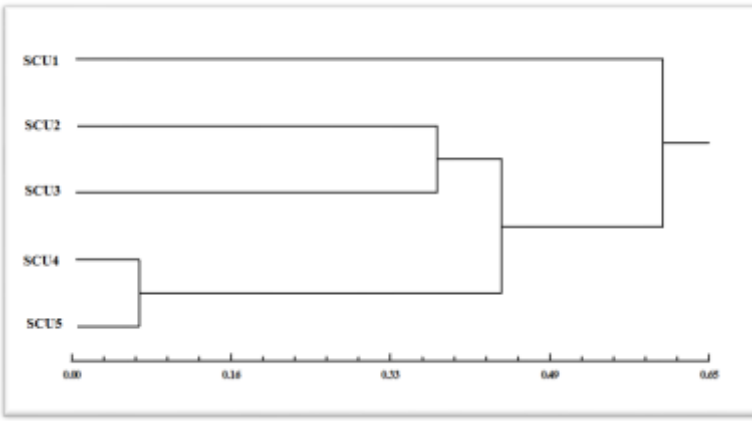

Figure (4): Dendrogramillustrated genetic fingerprint and relationship among *S. cerevisiae* isolates developed from ISSR data.

During dendrogram were constructed based on Nei and Lei (1979). Genetic distance using UPGMA cluster analysis and depicted genetic relationship ˆ ′e *S. cerevisiae* isolates showing two major clusters, the first cluster contained two main group, the first group contained SCU2 and SCU3 while the second group contained SCU4 and SCU5 formed another sub-cluster with low genetic distance 0.06899 and second cluster contained isolate SCU1. The characteristics of the individual genes are the result of the genetic material inherited by the individual of the parents in addition to the impact of the environment. In this study, the effect of the environment (dietary medium) on which the yeast was grown is evident through the results of the analysis of the RAPD using a random sequence of primers and ISSR using a sequence of specialized primers the total number of bands resulting from the RAPD analysis was 111 bands. The number of a polymorphic band was 101 bands and 9 unique bands as for the ISSR markers, it is giving 145 bands. The polymorphic band was 100 bands and the number of unique bands was 12 bands. Meaning that yeast isolates contained sequences own specialized indices rather than random indicators appeared in both markers RAPD and ISSR the extent of convergence between isolates SCU2 and SCU3, as well as near the isolates SCU4 and SCU5, as well as appeared in both markers in addition to phenotypic analysis that the isolate SCU1 is furthest from the other samples. Presence of yeast species on fruits can also be dependent on the geographical factors as well as the place from where the fruits are obtained [28] and their further post-harvesting treatment. In this study, we did not have information about the nature of the treatment suffered by fruit in their environment before they are used in the study. It may have been exposed to some chemical transactions that may have led to obtain mutations or delete or add to the rules of nitrogenous but it is clearly proved in the dendrogram that yeast isolated from different sources are very much different from each other, being, may be of different species or of different strains.

Conclusion

Presence of genetic diversity using RAPD and ISSR analysis as well as different observed colony morphological characteristics in the yeast strains from different fruit sources, it purposes a methodology for easy and quick isolation of yeast strains for both research and industrial analysis.

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