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Determination of genetic variance and the genetic dimension of cultivars of the bean genus Phasealus L. using RAPD - PCR indicators

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***Abstract*---**The current research aims to determine the DNA Finger Printing and Genetic Distance for 12 cultivars species of the genus Phasealus L Bean genus. Using RAPD-PRC Randomly Amplified Polymorphic DNA PCR indicators, which were extracted for DNA from young leaves of the cultivars, the purity and concentration regarding DNA have been measured with the use of a nanodrop device. Through the results obtained, the used primers were selected (804), including (478) general packages and (326) differentiated packages, and the occurrence of genetic fingerprints for most varieties characterized by some distinct packages amounting to (13) packages, including (8) unique packages and (5) An absent bundle, and the cultivar Brs - Pitanjo got the largest number of unique bundles, it owned (3) bundles. In contrast, the cultivars Brinco, Dellaregina, and massorn have been characterized to have the lowest number of bundles, amounting to (1) bundle. The absent bundles distinguished the two cultivars Brs - Pitanjo, BRs - Executive The highest number reached (2) bundles, while the Frasca variety recorded the lowest number of bundles reached (1) bundle. The study also showed that the values regarding the genetic dimension is in range of (0.016-0.375), while the lowest genetic dimension appeared between both cultivars (Siscresiatain Paleta and Massoru) and at a similarity reached (0.0 16) What is between these two studied categories. The highest genetic dimension of (0.375) was found between the two cultivars (BRS-

Executive and Lima bean), and this is the minor genetic similarity between the studied cultivars. It follows from this that the studied cultivars were characterized by the presence of genetic variation among them in addition to their unique and absent packages. The genetic dimension and genetic tree were determined Among the studied cultivars; the RAPD indicators showed high efficiency in determining the genetic dimension and genetic fingerprint of the studied bean species L. Phaseolus.

***Keywords*---**genus phaseolus, RAPD–PCR, genetic variance, genetic dimension, cultivars.

Introduction

The leguminous family (Leguminosae) Fabaceae includes the bean genus Phaseolus L. It is specified as one of the most significant crops due to the fact that its seeds include many proteins and sugary, starchy materials, and some vitamins. Its seeds are also used as a source of protein. Beans contain some amino acids, carbohydrates, and vitamins. The legume family is a large family that include 35 wild genera and about 300 species in Iraq, and some farmed species. Al-Samurai (2014).

Classification of Phaseolus

Kingdom: Plant
Division: Spermatophyta
Sub Division: Angiosperme
Class: D: Cotyledonae
Order: Fabales
Family: Fabaceae (Leguminosae)
Genus: *Phaseolus* L.
Itis (2014)

The bean genus is characterized by the low number of chromosomes 22 = 2n. The genetic study aims to find genetic variation and differences that accumulate in the organism and distinguish it from family members within the same taxonomic level. Karp et al. (1997), where the genetic study supported taxonomic studies, especially about races and species, and varieties are a real pillar of modern classification. One of the modern methods that can be relied upon in modern classification is the indicators of genetic material and its techniques, especially the genetic material DNA, because the genetic material is not affected by environmental conditions and is of fixed quantities in all cells in the plant—meteor (2020).

Through the developments that took place in molecular biology, the emergence of a new type of genetic indicators, which are DNA indicators, which are characterized by their ability to show differences and discrepancies in the inheritance of identical sequences of DNA between individuals. It is affected by environmental conditions and has been characterized by its comprehensiveness

and superiority over the indicators depending on analysis. Another benefit of the organism's protein content is its capability to identify vast numbers of various polymorphism variations, allowing it to detect even little variances. Al-Sakmani et al. (2018) Due to these characteristics of the DNA indicators and their broad areas of applications, the indicators were classified depending on the type of technologies used to find and detect them. RAPD indicators have been utilized in the presented research, where these indicators were described for the first time by Williams (1990), which are indicators based on PCR technology. Where the (Loci) sites on the DNA strand are duplicated using short random oligonucleotide primers, these primers consist of about ten nitrogen bases that link these primers to complementary sites on DNA strand, and often primers are random sequences with a high content of cytosine and guanine (G). - C), which generally ranges between 60-70% of its total bases, Omair (2009). Those indicators were distinguished by their precision, ease of use, and detection capability over the entire plant genome, as well as their inexpensive cost when compared to other indicators, Amir (2009). The indicators of RAPD - PCR are also fully dominant, as they do not distinguish between identical and divergent alleles. Early detection was allowed by Bertorelle and Barbujani (2005). The selection regarding the required genotypes and strains and the bean plant is one of the wide plants in genetic variations and the lack of molecular studies. Therefore, the current research aims to determine the genetic fingerprint and genetic dimension of some varieties of the genus Bean.

Materials and Methods

Plant Specimens Preparation

Leaf plant samples were collected in mid-April, where fresh young leaves were taken (4-5) weeks old and free of disease infections for each cultivated species. The young leaves were washed. DNA has been extracted from the young leaves regarding the examined varieties with the use of Kit technique that was acquired from Korean company FAVORGEN/ Biotech CORP according to (Shehab, 2020 and Al-Badrany, 2020) and then dried in a dark room at a temperature of (25) °C.

Genomic DNA Extraction

The Kit technique has been used for extracting DNA from young leaves of cultivars investigated. In addition, DNA has been examined and purified using Quality Control assays, which consist of 3 steps, the first determines the extracted DNA's concentration (in nanograms/microliter). The second step determines the purity regarding DNA Nuclear extracted through reading the absorbance with a Bio-Nano Drop spectrophotometer at wavelengths (260-280) nm, and the migration of DNA into an agarose gel is determined as indicated in Table1.

Table1: Genotypes that have been utilized in this research, along with their symbols

No.	Genus	Variety	Source
1	*Phaseolus vulgaris*	BRS. Executive	From local marker (Suaimania, Kurdistan Iraq)
2		Strick	From local marker (Mosul, Iraq), Netherlands
3		Gii-867	From local marker (Dohuk, Kurdistan Iraq)
4		Brinco	From local marker (Mosul, Iraq), Turkish.
5		Dellaregina	From local marker (Mosul, Iraq), Turkish.
6	*Phaseolus lanatus*	Brs-Pitanjo	From local marker (Erbil, Kurdistan, Iraq), Iranian
7		Scarlet runner bean	From local marker (Suaimania, Kurdistan Iraq)
8		Tabasco	From local marker (Suaimania, Kurdistan Iraq)
9		Lima beans	From local marker (Dohuk, Kurdistan Iraq)
10	*Phaseolus cocineus*	Siscresiatian palata	From local marker (Erbil, Kurdistan Iraq)
11		Masson	From local marker (Dohuk, Kurdistan Iraq)
12		Frasca	From local marker (Erbil, Kurdistan Iraq)

Performing RAPD-PCR reactions

Randomly Primer indicators supplied by the Korean company Macrogen were used, and their sequences can be seen in Table2.

Table2: Exhibits random prefixes that have been utilized in this work

Number	Primers	Sequences 5-3	Resource
1	OPA01	CAGGCCCTTC	Bukhari *etal.* (2015)
2	OPA03	ACTCAGCCAC	
3	OPA04	AATCGGGCTG	
4	OPA05	AGGGGTCTTG	Szilagyi *et al.*, (2011)
5	OPA07	GAAACGGGTG	
6	OPA09	GGGTAACGCC	
7	OPA10	GTGATCGCAG	
8	OPA11	CAATCGCCGT	
9	OPA17	CACCGCTTGC	
10	OPB10	CTGCTGGGAC	
11	OPC08	TGGACCGGTC	
12	OPG14	GGTGAGACC	
13	OPE06	AAGACCCCTC	

Table3 lists concentrations and quantities of amplification mixture as specified by the Korean Macrogen processor company's specifications.

Table3. Components of random amplification mixture

No.	Component	Concentration	Size (ml.)
1	$Mgcl_2$	25 mm	1
2	Primer	20 Pmol/ml	2
3	Master mix	2x	10
4	DNA template		2
5	Water		5
Total			20

The reaction components have been after that combined for five seconds in QLS-type Micro Centrifuge, after that, samples are placed into a Thermocycle device to complete the reaction or random amplification through utilizing the device's program for all subsequent reactions and all examined kinds Teng (2002). Following the process of amplification is completed, samples are prepared for electrophoresis through the mixing of (4) micro-liters of DNA Ladder with (2) micro-liters of the loading dyes. After adding loading dye to certain hole on one agarose side, (6.5) microliters of PCR products are removed and carried into the agarose pits with no dye since it has a distinct color. The gel has been transferred then immersed in water basin which contain distilled water 60 minutes following the transfer to eliminate excess dye traces. Then, the gel containing the PCR products was examined using a U.V. source Biometr, Germany Gel Documentation Trans illuminator Bands to determine the resulting bundle sizes.

Statistical Analysis

Evaluation regarding the discriminatory ability and efficacy of RAPD prefixes. For each initiator, the efficiency has been evaluated with the use of equation indicated via Grundman *etal.* (1995): Initiator efficiency = (No. of the packets in each one of the initiators / total No. of multiply packets in each one of the initiators) x100. As for discriminating capability, it has been identified depending upon the next correlation:

Discriminative power = (No. of the differentiated packets within each primer/total number of the divergent packets of all of the primers) x10

Genetic estimations

The values of the genetic dimension was estimated by converting the results of indicators for RAPD obtained in the gel into characterization tables by placing (1) when the bundle is present and (0) when the bundle is absent for finding the genetic relation between the studied varieties based on the equation mentioned by Nei and Lei (1979).

Results and discussion

The results regarding DNA isolation of studied Phaseolus species according to the kit method and using (13) random starters for young leaves in order to avoid the high content of phenols, sugars, and other secondary metabolic compounds that will interfere and affect the DNA when extracted (Tahir, 2015). Using a large

number of primers in RAPD indicators specifies a higher level of precision in measuring genetic dimension. Various researchers have verified the possibility of identifying the biggest area of genome (Figure 1-13). (Al-Qaisi, 2013, Al-Assi, 2002, and Al-Zuhairi, 2014). Table (4) shows the findings of the primers, which revealed several forms of bundles. The primers identified (134) sites on the genome of samples at rate of (10) bundles for every one of the initiators, of which (39) have been general sites with a (3.9) rate for each one of the initiators and (56) sites with rate of (4) packets per initiator, with primers (OPA06, OPA-3, OPA-1) having the highest count of chosen sites, which reached (9) sites. The initiator OPA-11 had the lowest production of sites and amounted to (4) sites, and this corresponds to some extent with the study of Bukhair, et al., 2015. Which mentioned that the initiator OPA-1, OPA-3 produces the largest number of sites in addition to what was found by (Muzher *et al.*, 2014 and Al-Sakmani *et al.* 2018)

RAPD indicators were used because they are cost-effective and straightforward, and they are the preferred method for less advanced laboratories (Yaday et al. (, 2014). The young leaves were also used to avoid the high content of phenols, sugars, and other secondary metabolites that will interfere and affect the DNA when extracted. (Tahir, 2015). Moreover, the total bands chosen by those sites shown in Table (4) is (804) number of total fragments., of which (478) are the number of original fragments and (326) are polymorphic fragments, where the initiator (OPA - 3) produced the highest number of packages produced, which reached (85). The initiator (OPA - 11) had only one bundle and the fewest number of bundles (29), and the overall Variation Ratio of the created primers was 59.47%. The indicators of the RAPD depend on the foundations, which represents number of the bundles, which show the the genome of any model, and then indicates to the no. of the sites which the initiator finds then connects, and that the differences in the types, numbers, and sizes of bundles in the varieties of the studied bean genus are due to the initiator's ability to determine. Finding genetic variation between species, depending on the sequence of the nitrogenous bases of the DNA. The development of induced mutations or self-mutations which modify distance between the sites which arise spontaneously throughout the evolutionary process of the living organisms causes variations. Differences could have haened as a result of replacement, addition or deletion of the sites that are related with the initiator Al-Karkhi (2018) and Al-Sakmani *et al.* (2018).

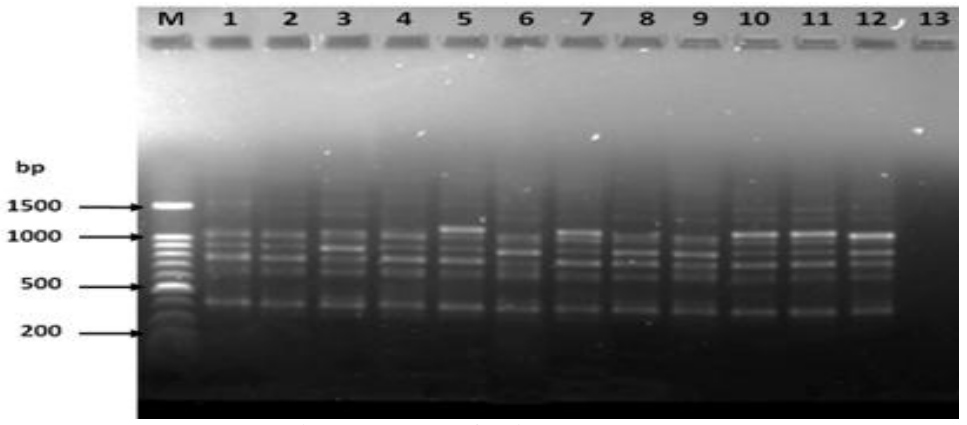

Figure1:PCR of Primer OPA1

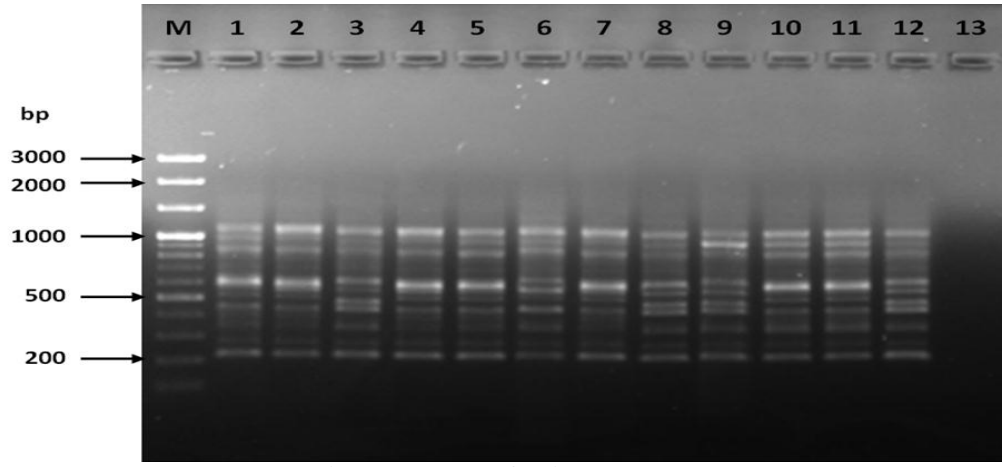

Figure2: PCR of Primer OPA3

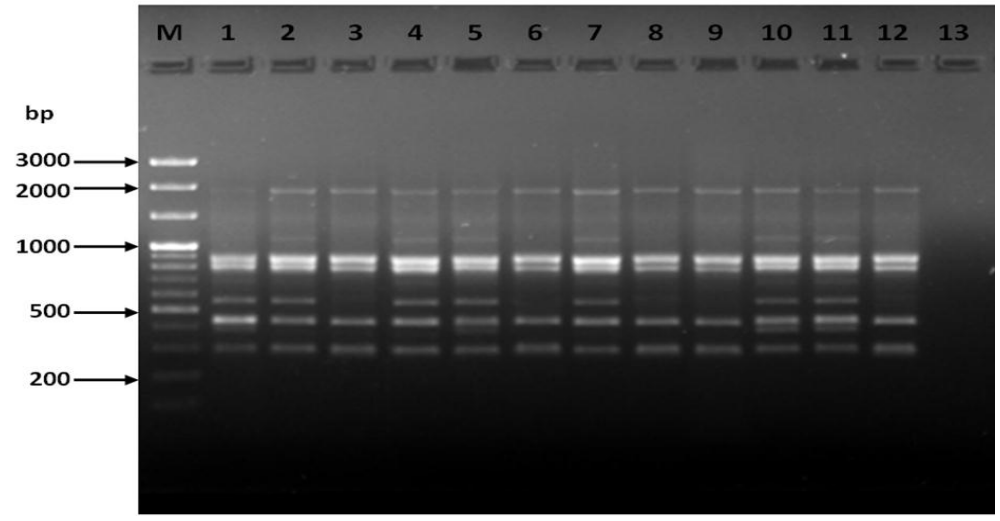

Figure3: PCR of Primer OPA4

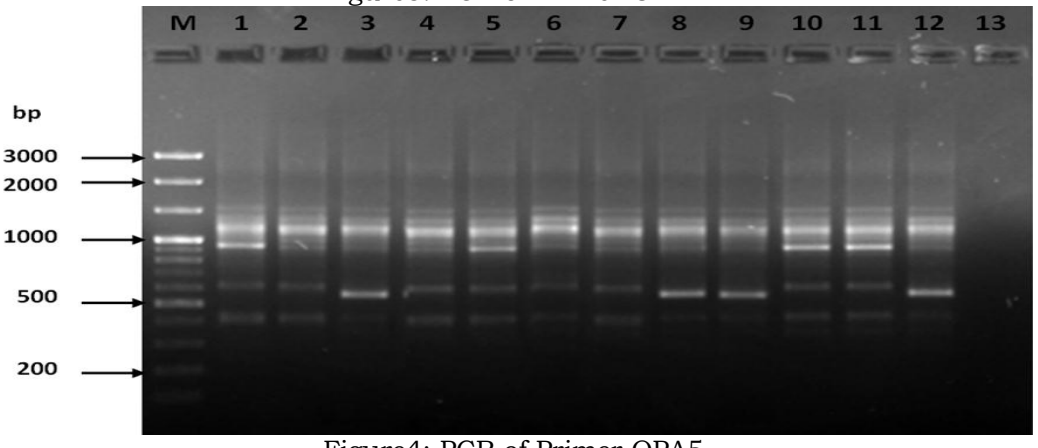

Figure4: PCR of Primer OPA5

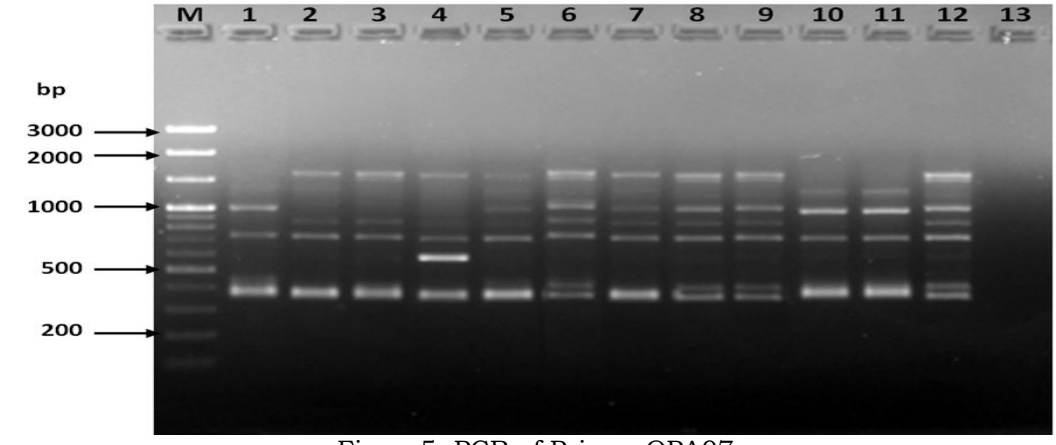

Figure5: PCR of Primer OPA07

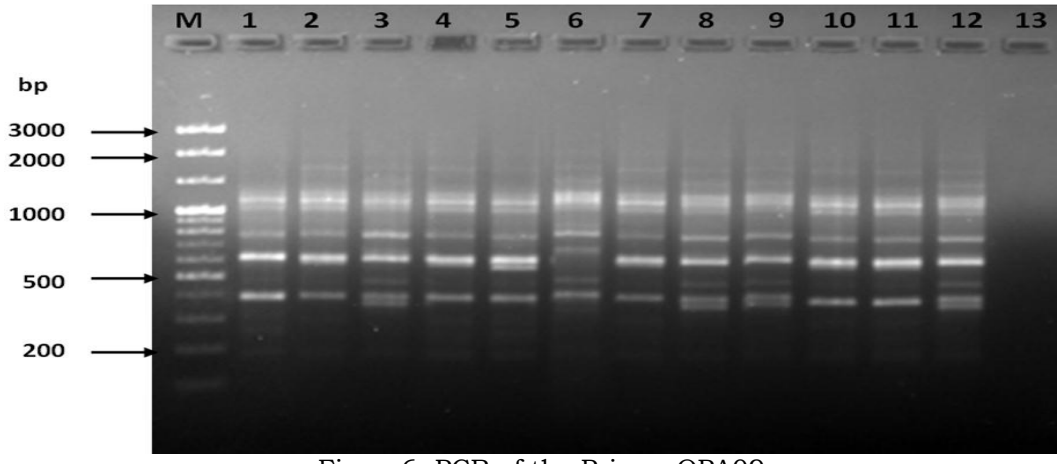

Figure6: PCR of the Primer OPA09

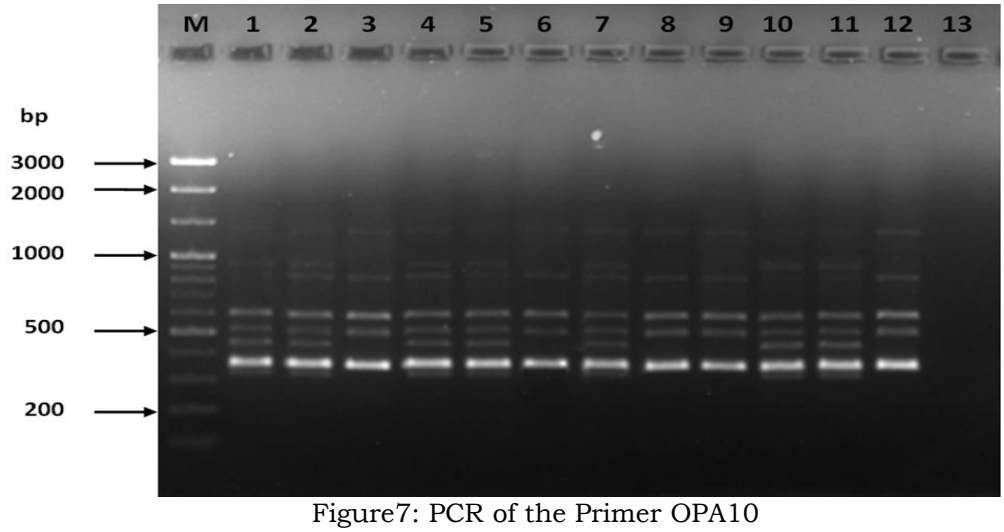

Figure7: PCR of the Primer OPA10

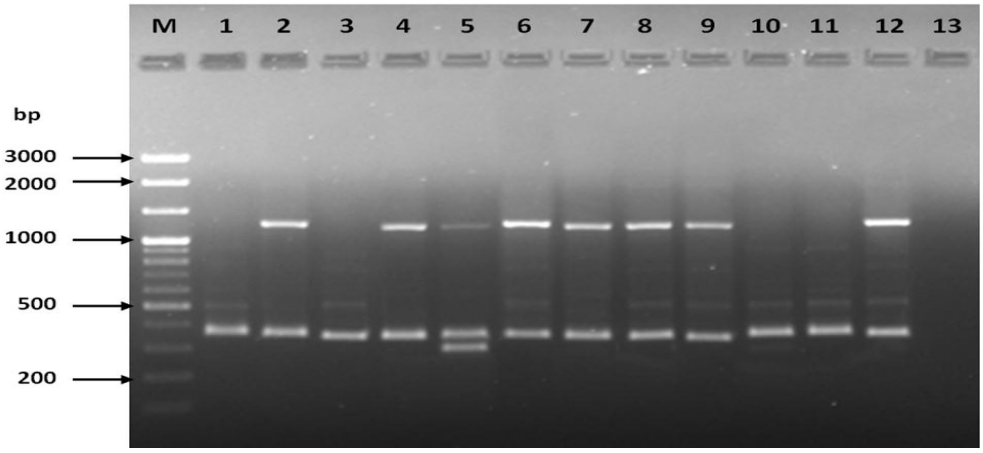

Figure8: PCR of the Primer OPA11

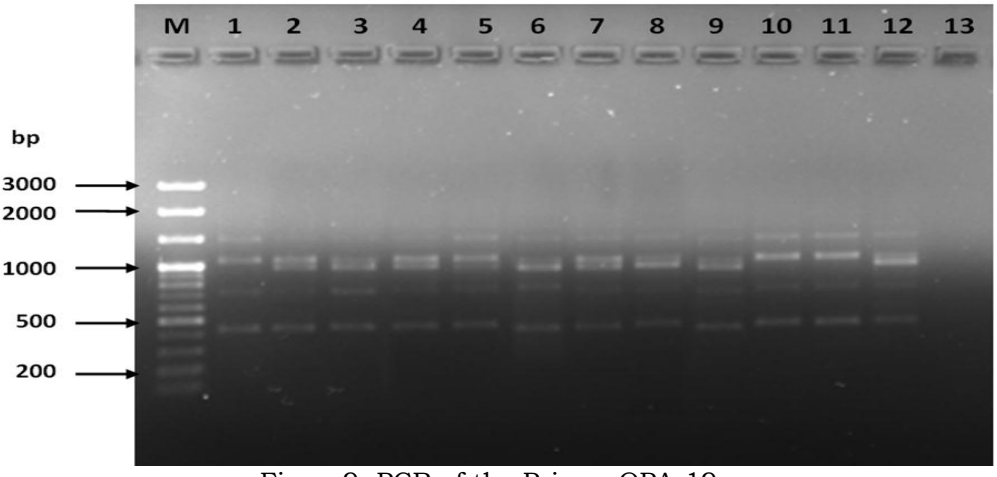

Figure9: PCR of the Primer OPA-19

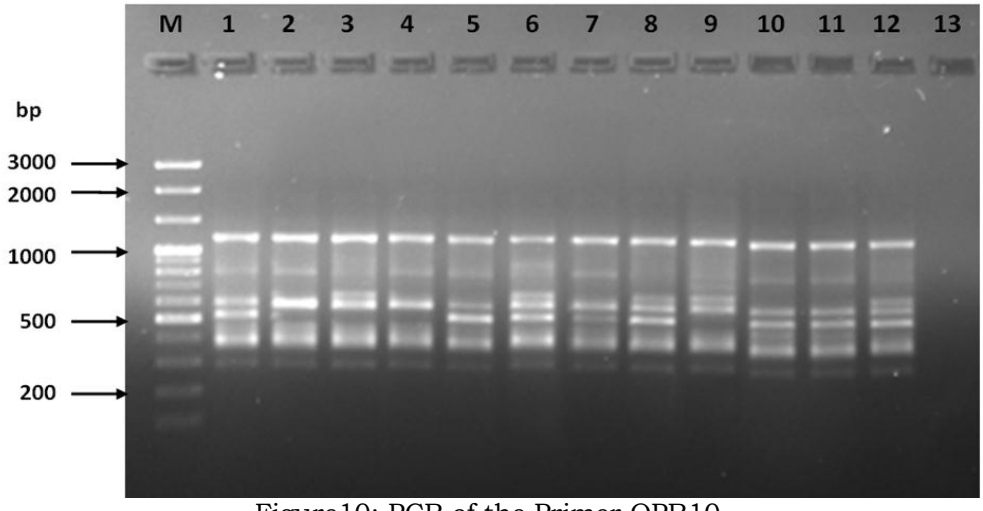

Figure10: PCR of the Primer OPB10

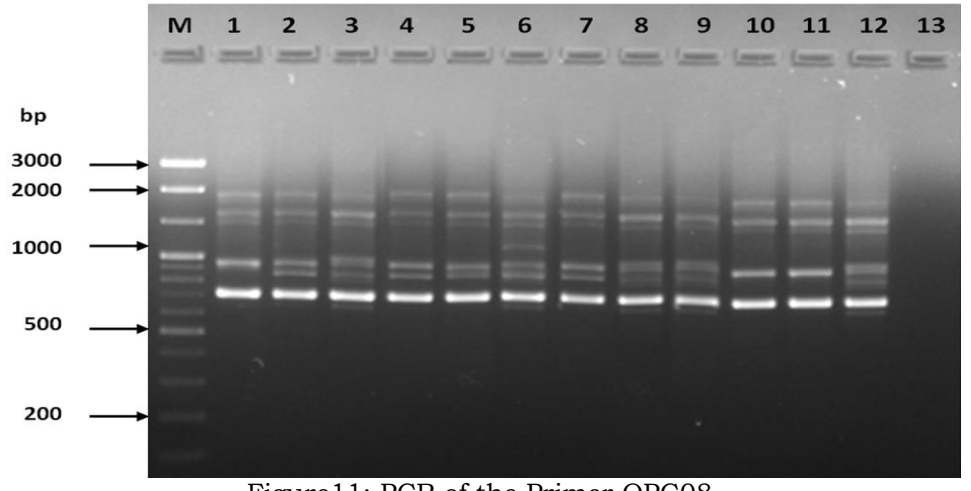

Figure11: PCR of the Primer OPC08

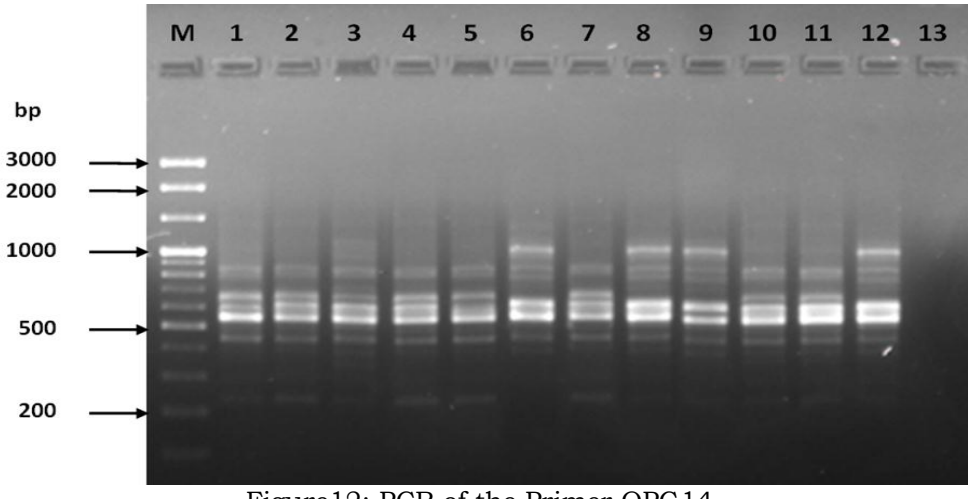

Figure12: PCR of the Primer OPG14

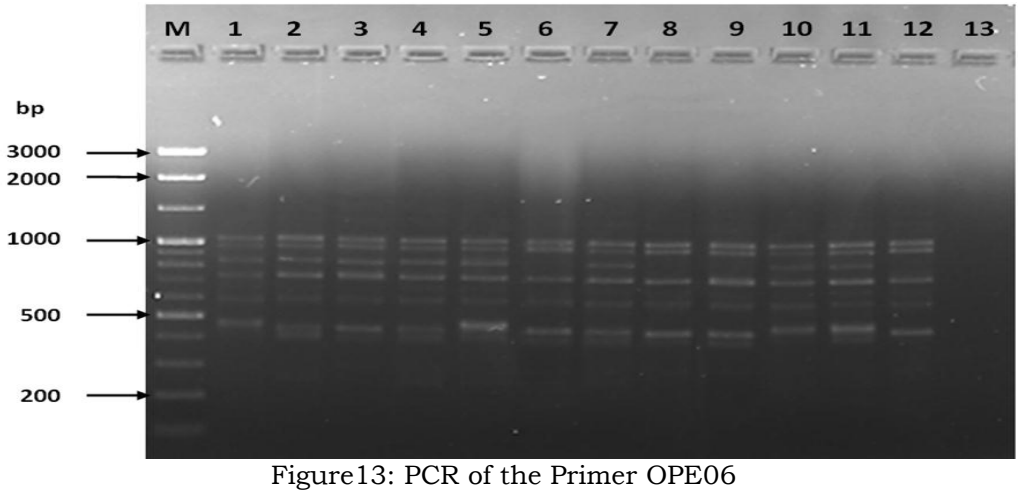

Figure13: PCR of the Primer OPE06

This was stated by Bukhari *et al.* (2015) when studying genetic variations regarding bean cultivars using RAPD indicators with (19) random prefixes. The existence of distinctive bundles (Unique band and Absent Bands) distinguished a few of the examined types, as seen in Table (18). The total number of diverse bundles formed by the prefixes in this investigation was (8) unique bundles, (13) distinct bundles, and (5) absent bands. These bundles are among the most important diagnostic traits adopted in the differentiation of varieties and species, as we find that a unique bundle distinguished the Massoru cultivar at the initiator OPE-6 and the molecular size of 10, bp, and cultivar Brinco has been distinguished via a unique bundle at the initiator OPA-7 at a molecular size of 540bp and in the cultivar Brs-Pitanjo The unique band appeared in the primers (OPA-19, OPB-610, and OPC-8) at molecular sizes (780, 900, and 1080) bp, respectively, while the BRs-Executive cultivar was characterized by a unique band at the primers (OPA-1 and OPE-6).) at molecular sizes (1500 - 470) bp. The cultivar Dellaregina showed a unique initiator band (OPA-11) with a molecular size of 310bp. The observation of these bundles in varieties and their appearance is one of the most important distinguishing characteristics that distinguish them from the rest of the other varieties, as the appearance of these bundles in one class without another indicates the presence of that sequence in the genetic DNA of that variety without the other varieties. Bukhari *et al.* (2015). It may also indicate a mutation at a specific site which result in the recognition of initiator as well as the the emergence of the (unique) distinctive bundle Tingey (1993) and Al-Asi (2002).

As for the absent bands and their appearance in a particular cultivar and not in other studied cultivars, it is an indication and indicator of the distinctiveness of this cultivar. The OPA-1 primer showed an absent band for Frasca at a molecular size of 900bp, and cultivar Brs-Pitanjo has been distinguished via the absent bands for the primers OPB-10 and OPA. -1 at molecular sizes 590-680 bp, while BRs-Executive cultivar showed absent bands at OPA-1 and OPE-6 primers, and at molecular sizes 1500 and 470 bp as shown in Table (18), where the presence of these bands is a distinguishing formula for the cultivar. It may indicate taxonomic importance, and the reason may be due to the lack of suitable binding sites for the initiator on the DNA strand of the cultivar, as this could be used to construct differences between taxa and species. Al-Ablish (2012) and Shehab (2020).

The primers' efficiency also differed in terms of displaying the variance between the tested types, with the initiator OPA-11 being the least efficient at 3.61 and the initiator OPA-3 being the most efficient at 10.59, as seen in Table (18). As for the differentiating ability of the prefixes, the initiator OPA-1 was distinguished. The highest discriminating ability was 11.4, while the lowest discriminating ability was for the OPA-9 initiator, which was 3.68, as the initiator efficiency increases with an increase in the amount of the resulting packets Hormaza (2018) and Khierallah (2014). The genetic distance has been evaluated from results regarding interactions of RAPD indicators between genotypes included in the study for the varieties of the genus Phaseolus L. and through the genetic dimension matrix that has been listed in Table16. The genetic dimension values ranged (0.016-0.375), where the highest genetic dimension was (0.375) between the two cultivars B.R.s - Executive and Limabea, while the minimal genetic dimension has been between the two cultivars Siscresiation Paleta and Massorn and reached (0.016). In

contrast, the values of the genetic dimension ranged for the rest The varieties between these values, as the genetically separated varieties are the ones that share the least number of bundles with each other due to the presence of differences in the sequence of nucleotides in the genome of these varieties. Here, the role of using different primers that target regions of the genome will appear, and thus a difference will appear between the varieties of the studied species. According to the sequence of the initiator and the degree of variation between the genomes of the studied species. Yassin (2011) Al-Zuhairi (2014) Al-Sakmani *et al.* (2018) Al-Badarni (2020).

Also, the order of the varieties and depending on genetic dimension values in genetic kinship tree or dendrogram (Figure 14) based upon genetic range to which major groups are linked and, thus, existence of section regarding varieties in a certain group specifies level of the genetic similarity of these varieties in the group. El kichaoui (2013) and Abboud (2014) and Shehab (2020). The Dendrogram group was established for the studied varieties using cluster analysis, as it has been discovered that it was divided to three major groups; the first main group was divided into two subgroups, as can be seen from figure (14), while the second main group included only one category, which is B.R.s. Executive, the third main group also included the cultivar scarlet runner bean, as shown in Figure (14). Through those results that we have obtained, it has been noted that there has been degree of similarity, difference, or genetic variance between the varieties of the studied species, and this shows that anatomical phenotypic traits have an impact on knowing the genetic variance between The studied cultivars, as well as the RAPD indicators, show that the anatomical phenotypic traits are due to the expression of the genes responsible for them. However, differences may occur between the cultivars and the lack of evidence for any phenotypic trait.

This is due to environmental factors on the one hand and genetic interference on the other hand, which makes the confirmation of phenotypic traits challenging to conclude from all this that it is not possible to rely entirely on those traits in isolating and diagnosing varieties and species. Thus, it makes reliance on genetic indicators based on DNA the decisive factor in identifying and diagnosing species and varieties. Because it is not affected by any external factor and it is fixed and present in all cells and all stages of growth, and this agrees with many researchers that have utilized the RAPD indicators in studying genetic variation of many plant varieties, for example, the study of Al-Asi (2002) random initiator. In its use in the analysis of genetic diversity, where (38) random starters were used, and (16) of them were able to find the genetic fingerprint of ten genotypes of barley, and in the study of Muhammad (2010) to analyze the genetic variance of oranges using (19) random starters, as well as Shehab (2020), determine Genetic variance for some apple and pear cultivars using (12) random starters. In the study of Al-Badrani (2020) genetic variance of berry varieties was studied using (12) random starters. As for the bean plant, Tahir (2015) study of genetic variance for 10 varieties of farmed broad beans. In the city of Sulaymaniya, utilizing 18 random starters in the RAPD indicators, most of these primers showed genetic variance, as well as the study of Basheer (2012), where the RAPD indicators have been utilized for the purpose of finding genetic variance of 26 varieties of Beans in Palestine using (26) random starters. Al-Ghamdi (2009), where eight cultivars of

Beans were used and (14), were used as a starter and showed their efficiency in finding the genetic dimension of those cultivars in the Kingdom of Saudi Arabia— reducing the use of RAPD indicators to find the genetic variance and the genetic dimension between them. Moreover, the study of Bukhari *etal.* (2015) in determining genetic variance of some cultivars of beans by using (19) random initiators to assess genetic differences and the relationship between cultivars stated that RAPD techniques are useful in distinguishing between cultivars of Phaseolus bean species.

Table 4: Primer 1

bp	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
1500	1	0	0	0	0	0	0	0	0	0	0	0
1266	0	0	0	0	0	0	0	0	0	1	1	0
1161	0	1	1	0	0	1	0	0	1	1	1	0
1066	0	0	0	0	1	0	1	0	0	1	1	1
900	1	1	1	1	1	1	1	1	1	1	1	0
800	1	1	1	1	1	1	1	1	1	1	1	1
680	1	1	1	1	1	0	1	1	1	1	1	1
560	1	1	1	1	1	1	1	1	1	1	1	1
390	1	1	1	1	1	1	1	1	1	1	1	1

Table 5 :Primer 2

bp	P 1	P 2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P 12
1270	1	1	1	1	1	1	1	1	1	1	1	1
1000	1	0	0	0	1	1	0	1	1	1	1	1
850	1	1	1	1	1	1	1	1	1	1	1	1
600	1	1	1	1	1	1	1	1	1	1	1	1
520	0	0	1	0	0	1	0	1	1	0	0	1
450	0	0	1	0	0	0	0	1	1	0	0	1
430	1	1	1	1	1	1	1	1	1	1	1	1
345	1	0	1	0	0	1	0	1	1	1	1	1
220	1	1	1	1	1	1	1	1	1	1	1	1

Table 6: Primer 3

bp	P1	P2	P 3	P4	P5	P6	P 7	P 8	P9	P10	P11	P12
1820	1	1	1	1	1	1	1	1	1	1	1	1
1000	0	1	0	1	0	0	1	0	0	1	1	0
840	1	1	1	1	1	1	1	1	1	1	1	1
800	1	1	1	1	1	1	1	1	1	1	1	1
550	1	1	0	1	0	0	1	0	0	1	1	0
430	1	1	1	1	1	1	1	1	1	1	1	1
300	1	1	1	1	1	1	1	1	1	1	1	1

Table 7 : Primer 4

bp	P1	P 2	P 3	P4	P5	P6	P7	P8	P 9	P10	P11	P 12
1450	1	1	1	1	1	1	1	1	1	1	1	1
1270	1	1	0	1	1	1	1	0	0	0	0	1

1090	1	1	1	1	1	1	1	1	1	1	1	1
920	1	0	0	0	1	1	0	0	0	1	1	0
600	1	1	0	1	1	1	1	0	0	1	1	0
515	0	0	1	0	0	0	0	1	1	0	0	1
410	0	0	0	0	0	0	0	1	1	0	0	0
420	1	1	0	1	1	1	1	0	1	1	1	0

Table 8 :Primer 5

bp	P 1	P 2	P 3	P4	P5	P6	P 7	P8	P 9	P10	P11	P12
1450	0	1	1	1	1	1	1	1	1	0	0	1
1165	0	0	0	0	0	0	0	0	0	1	1	0
1000	1	0	0	0	1	1	1	1	1	1	1	1
740	0	1	1	0	0	1	1	1	1	0	0	1
718	1	1	1	1	1	1	1	1	1	1	1	1
540	0	0	0	1	0	0	0	0	0	0	0	0
390	0	0	0	0	0	1	0	1	1	0	0	1
366	1	1	1	1	1	1	1	1	1	1	1	1

Table 9: Primer 6

bp	P1	P 2	P 3	P 4	P5	P6	P7	P8	P9	P 10	P 11	P12
1524	0	1	0	1	0	0	1	0	0	0	0	0
1180	1	1	1	1	1	1	1	1	1	1	1	1
800	1	1	1	1	1	1	1	1	1	1	1	1
600	1	1	1	1	1	1	1	1	1	1	1	1
420	0	0	1	0	0	1	0	1	1	0	0	1
376	1	1	1	1	1	1	1	1	1	1	1	1
360	0	0	1	0	0	0	0	1	1	0	0	1

Table 10 :Primre 7

bp	P1	P 2	P3	P 4	P5	P6	P7	P 8	P9	P10	P11	P12
1400	0	1	1	1	0	1	1	1	1	0	0	1
970	1	1	0	1	0	0	1	0	0	1	1	0
810	0	1	1	1	1	1	1	0	1	0	0	1
600	1	1	1	1	1	1	1	1	1	1	1	1
500	1	1	1	1	1	1	1	1	1	1	1	1
450	1	1	0	1	1	0	1	0	0	1	1	0
340	1	1	1	1	1	1	1	1	1	1	1	1

Table 11 : Primer 8

bp	P1	P2	P 3	P4	P5	P6	P 7	P 8	P9	P 10	P11	P12
1222	0	1	0	1	1	1	1	1	1	0	0	1
500	1	0	1	0	0	1	0	1	1	1	1	1
365	1	1	1	1	1	1	1	1	1	1	1	1
310	0	0	0	0	1	0	0	0	0	0	0	0

Table 12 : Primer 9

bp	P 1	P 2	P3	P4	P5	P6	P 7	P8	P9	P10	P11	P12
1400	1	0	0	0	1	1	0	1	0	1	1	1

1120	1	1	0	1	0	0	1	0	0	1	1	0
1000	0	1	1	1	0	1	1	1	1	0	0	1
780	0	0	0	0	0	1	0	0	0	0	0	0
700	1	0	1	0	0	0	1	1	1	1	1	1
440	1	1	1	1	1	1	1	1	1	1	1	1

Table 13 : Primer 10

bp	P 1	P2	P3	P4	P5	P 6	P 7	P8	P 9	P10	P11	P12
1147	1	1	1	1	1	1	1	1	1	1	1	1
900	0	0	0	0	0	1	0	0	0	0	0	0
850	1	1	1	1	1	1	1	0	0	1	1	0
650	0	0	1	1	0	1	0	1	1	0	0	1
590	1	1	1	1	1	0	1	1	1	1	1	1
530	1	0	1	0	1	0	1	1	1	0	0	1
380	1	1	1	1	1	1	1	1	1	1	1	1
300	1	1	1	1	1	1	1	1	1	1	1	1

Table 14; Primer 11

bp	P1	P2	P3	P 4	P5	P 6	P 7	P8	P9	P 10	P11	P 12
1885	1	1	1	1	1	1	1	1	0	1	1	1
1538	1	1	1	1	1	1	1	1	1	1	1	1
1400	1	1	1	1	0	1	1	0	1	0	0	1
1080	0	0	0	0	0	1	0	0	0	0	0	0
950	1	1	1	1	1	1	1	1	1	1	1	1
820	0	1	1	1	1	1	1	1	1	0	0	1
700	1	1	1	1	1	1	1	1	1	1	1	1

Table 15: Primer 12

bp	P 1	P2	P3	P4	P 5	P6	P 7	P8	P9	P 10	P 11	P12
1000	0	0	0	0	0	1	0	1	1	0	0	1
860	1	1	1	1	1	1	1	1	1	1	1	1
680	1	1	0	1	0	0	1	0	1	0	0	0
600	0	1	1	0	0	1	0	1	1	1	1	1
560	1	1	1	1	1	1	1	1	1	1	1	1
450	1	1	1	1	1	1	1	1	1	1	1	1

Table 16: Primer 13

bp	P 1	P2	P 3	P4	P5	P 6	P 7	P8	P9	P10	P 11	P 12
1022	1	1	1	1	1	1	1	1	1	1	1	1
950	1	1	1	1	1	1	1	1	1	1	1	1
850	1	1	1	1	1	0	1	0	0	1	1	0
710	1	1	1	1	1	1	1	1	1	1	1	1
610	0	0	0	0	0	0	0	0	0	0	1	0
580	1	1	1	1	1	1	1	1	1	1	1	1
470	1	0	0	0	0	0	0	0	0	0	0	0
430	0	1	1	1	1	1	1	1	1	1	1	1
350	0	1	0	1	0	1	1	0	1	0	1	0

Table 17:Genetic similarity

	P 1	P2	P 3	P4	P5	P 6	P7	P 8	P9	P 10	P 11	P12
P 1	1											
P2	0.824	1										
P3	0.766	0.833	1									
P 4	0.831	0.962	0.809	1								
P5	0.873	0.868	0.781	0.868	1							
P6	0.755	0.880	0.838	0.803	0.806	1						
P 7	0.857	0.855	0.820	0.955	0.893	0.800	1					
P8	0.763	0.761	0.909	0.766	0.784	0.855	0.779	1				
P 9	0.687	0.811	0.911	0.788	0.761	0.873	0.814	0.942	1			
P10	0.883	0.833	0.784	0.809	0.875	0.779	0.850	0.757	0.750	1		
P11	0.870	0.820	0.778	0.812	0.861	0.783	0.850	0.761	0.754	0.984	1	
P12	0.767	0.779	0.910	0.770	0.787	0.871	0.811	0.955	0.928	0.761	0.750	1
	0.807	0.833	0.831	0.834	0.832	0.823	0.853	0.821	0.820	0.824	0.820	0.826

Table 18: Genetic distance

	P1	P 2	P3	P 4	P 5	P6	P7	P 8	P9	P10	P 11	P 12
P 1	0											
P2	0.193	0										
P3	0.266	0.183	0									
P 4	0.185	0.039	0.212	0								
P 5	0.136	0.141	0.247	0.142	0							
P6	0.281	0.116	0.177	0.219	0.216	0						
P7	0.154	0.046	0.198	0.046	0.113	0.223	0					
P 8	0.270	0.273	0.095	0.266	0.243	0.157	0.249	0				
P9	0.375	0.209	0.093	0.238	0.273	0.249	0.206	0.060	0			
P10	0.124	0.183	0.243	0.211	0.133	0.250	0.163	0.278	0.288	0		
P11	0.139	0.198	0.251	0.208	0.150	0.245	0.163	0.273	0.282	0.016	0	
P12	0.265	0.250	0.094	0.261	0.239	0.138	0.209	0.046	0.075	0.273	0.287	0

Table 19:Results of primers used in RAPD reactions

Number	Primers	No of locations has produced	No. of original Location	No. of variation location	Total No. of the fragments for primer	Number of the original fragment	No. of variation locations	No. of unique fragments	No. of absent fragments	Percentage of Variations
1	OPA-1	9	3	6	72	36	36	1	2	66.66
2	OPA-3	9	5	4	85	60	15	0	0	44.44
3	OPA-4	7	5	2	73	60	13	0	0	28.57
4	OPA-5	8	2	6	59	24	35	0	0	75.00
5	OPA-7	8	2	6	56	24	32	1	0	75.00
6	OPA-9	7	4	3	60	48	12	0	0	42.85
7	OPA-10	7	3	4	65	36	29	0	0	57.14
8	OPA-11	4	1	3	29	12	17	1	0	75.00
9	OPA-19	6	1	5	42	12	30	1	0	83.33
10	OPB-10	8	3	5	70	36	34	1	1	62.50
11	OPC-8	7	3	4	65	36	29	1	1	57.14

12	OPG-14	6	3	3	53	34	17	0	0	50.00
13	OPE-6	9	4	5	75	48	27	2	1	55.00
	TOTAL	95	39	56	804	478	326	8	5	

Table 20. The unique, absent fragment, primer efficiency, and discriminating ability

No. Primer	Primers	Molecular size (Mbp)	Unique (U) and Absent (A) Premium fragment																						Primer efficiency (%)	Discriminatory ability of the primer (%)
			P1		P2		P3		P4		P5		P6		P7		P8		P9		P10		P11		P12			
			A	U	A	U	A	U	A	U	A	U	A	U	A	U	A	U	A	U	A	U	A	U	A	U		
1	OPA-01	1550-390	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	8.96	11.4
2	OPA-03	1270-220	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10.57	4.60
3	OPA-04	1820-300	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9.08	3.99
4	OPA-05	1450-420	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7.34	10.74
5	OPA-07	1450-366	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.97	9.82
6	OPA-09	1524-366	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7.46	3.68
7	OPA-10	1400-340	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8.08	8.90
8	OPA-11	1222-310	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.61	5.21
9	OPA-17	1400-440	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	5.22	9.20
10	OPB-10	1147-300	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	8.71	10.43
11	OPC-08	1885-700	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	8.08	8.90
12	OPG-14	1000-450	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.59	5.21
13	OPE-06	1222-350	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	9.33	8.28
Total			1	2	1	0	0	0	0	0	0	0	2	3	0	0	0	0	1	0	0	0	0	0	1	0	100%	100%

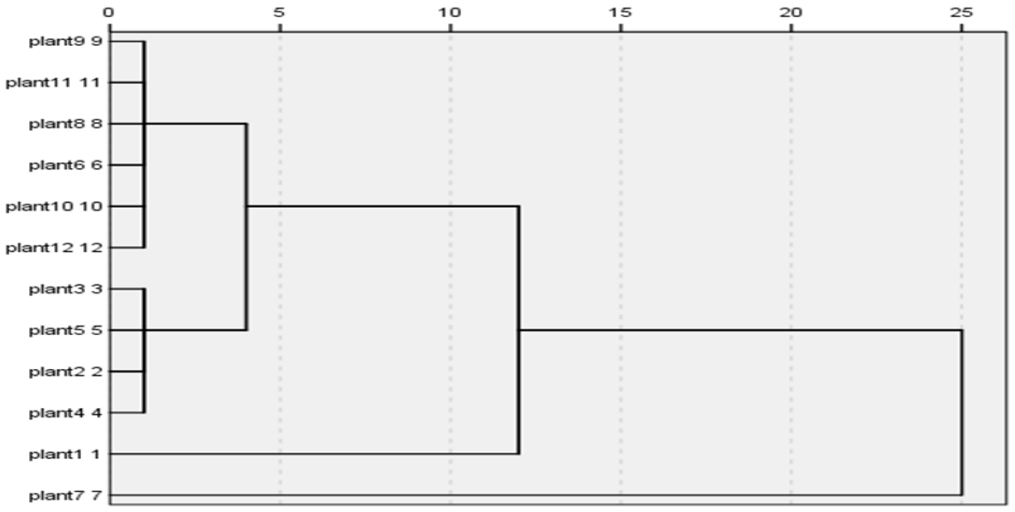

Figure14: Dendrogram using average similarity (between plants) rescaled distance cluster combine

Conclusion

The research paper indicates that the primers used produced (804) packages of them (478 normal bundles and (326) mixed bundles, the cultivar Brs - Pitanjo got

the largest number of unique bundles, The genetic dimension and genetic tree were determined Among the studied cultivars**.**

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