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Using Phenotypic And Molecular Indicators Rapd-Pcr To Evaluate The Performance And Genetic Dimension Of A Number Of Genotypes And Their Individual Hybrids In The Chickpea Plant Cicer Arietinum L

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Abstract

The study included (6) new genotypes of chickpea crop introduced from the International Center for Agricultural Research in the Dry Areas (ICARDA). The six genotypes were planted in the season (2018) to self-pollinate them for one season to confirm their purity, and then cross-hybrid between distinct varieties In its traits, which can be collected in the individuals of the first generation and in the season (2019), the Diallel Meting Design (AA) was carried out without reverse hybrids and self-pollination according to the second method (random model) according to Griffing (1956) to obtain (15) An individual hybrid as well as the parents' self-pollinating seeds of (6). In the season (2020), the experiment was applied in one of the farmers' fields in Dhi Qar city by using a complete random block design in three replicates to assess the genotypes (parents + hybrids) in order to conduct a phenotypic and molecular study in which two indicators based on PCR technology were used, which are indicators The RAPD by using custom primers from the RAPD index to determine the genetic correlation between the genotypes of quantitative traits (flowering date, plant height, number of pods / plant, number of seeds / pod, weight of 100 seeds and seed yield). According to the present results, it was found that it is possible to use RAPD indicators to evaluate the genotypes of chickpea plants into groups and estimate the genetic dimension between them, which is directly related to the molecular genetic dimension and the phenotypic genetic dimension. The father (1) and (5) and the hybrid (1×5) showed the largest genetic dimension, while the father (3) showed the lowest genetic dimension depending on the results of phenotypic and RAPD indicators, and these two parents and the hybrid were the most superior in most of the studied traits, as were the indicators of RE- RAPD is efficient in identifying (25) genetic mutations, as these mutations are a diagnostic genetic fingerprint

for most parents and a function of the presence of specific sites, especially for parents, in the parents' genome through the use of (7) primers.

Keywords: Genetics; Parents; Hybrids; Chickpeas.

Introduction

Chickpea plant, Cicer arietinum, is one of the main and important crops in the Middle East since (7500-6800) BC [1]. It is a field crop rich in proteins, vitamins and important mineral elements such as phosphorous and copper, and this is the reason why it is an important and preferred popular food, as the proportion of protein Chickpea seeds are very high compared to wheat, rice, corn, etc., reaching (21%) [2]. In addition, its seeds contain a high percentage of amino acids such as tryptophan, lysine, carbohydrates, fats and mineral salts [3]. The amount of the final yield of chickpeas is affected by many factors, including the genetic factor, the growing season and various agricultural processes. Many studies indicated that the narrow genetic base in the genetic resources was the reason for the limited success through the final increase of the yield [4], the [5] indicated to the importance of these important genetic resources in improving the quality and quantity of the crop, both phenotypically and molecularly[16] , Phenotypic indicators are the most important ways to study the genetic relations between a group of varieties, and there are many researches that have been conducted and proven the effectiveness of phenotypic indicators by showing the differences between different varieties that negatively affect the results of those indicators, which called us to move towards those indicators, which are more stable and less affected by influences. Environmental indicators [6], so that the concept of phenotypic indicators is one of the best, simplest and oldest methods and is the basis for entering the indicators that have been relied upon since antiquity and until now, and they are indicators seen with the naked eye, and these indicators are the basis for studying the genetic variation of plants [7], the development of molecular indicators has enabled researchers to overcome the obstacles they faced through the use of previous traditional methods has provided many ways in terms of speed, effort, accuracy and time by selecting the best of them and early for genetic performance [8] [17], the technique of PCR was discovered in the past two decades, which helped many researchers to derive different indicators that depend directly on this technology. That contributed to the evaluation of genetic strains and their identification through genetic fingerprint and the identification of the genes responsible for the important traits in the plant. The indicators of the RAPD were used for this work as a result of its

superiority in terms of accuracy, ease and detection with the widest area of the plant genome and low cost compared to other indicators.

Materials and methods

(6) Chickpea plant genotypes were obtained from ICARDA and (15) individual hybrids resulting from these parents. The total (21) genotypes were planted in a farmer's field in Dhi Qar city according to a randomized complete block design with three replications, which included The sector included (21) experimental units to which parents and camels were randomly distributed. Parents and camels were planted with lines at the rate of one line for each of the fathers and camels, and 10 seeds were planted for each line with a length of 3 m, and the distance between one seed and another was 0.2 m, and the distance between one line and another was 0.75 m. It has plowing, settlement and crop service operations in accordance with the recommendations followed. The chemical fertilizer NPK was added at an amount of 400 kg. Hectares after plowing, and the quantitative characteristics related to growth and yield characteristics were taken [5] [18]. Samples were collected from plants two months after the date of planting from the parents and camels as they were taken (6-4) Young leaves from the developing top were placed in special known bags and transferred to the laboratory directly to conduct the process of isolating DNA from them, and the analyzes and measurement, concentration and purity of DNA were carried out in the molecular laboratory of the College of Science at Al-Mustansiriya University.

Sample preparation:

The seeds of (21 genotypes), which included pure strains and their half hybrids, were planted in plastic pots. When the plant reached a height of (4-6), leaves were transferred to the Molecular Biological Laboratory of the Department of Life Sciences at Al-Mustansiriya University, and laboratory experiments were conducted to extract the genetic material DNA from it.

DNA Genomic DNA Isolation: DNA was isolated from young chickpea leaves using CTAB and the method mentioned by [9], and based on the principles set by [10].

Solutions used for DNA isolation:

1- Extraction Buffer: The extraction solution consists of:

- 1,4 molar Nacl
- 100 mM Tris-Hcl
- 20 mM Na2 EDTA

- 2% CTAB and sterilized by sterilizer, according to [11].

To prepare 200 ml of the extraction solution, weighing 4 g of CTAB, add to it 56 ml of sodium chloride solution (5 molar), which is prepared with a weight of (29.22) g of salt (Nacl) and dissolve it in (100 ml) distilled water, then add 10 ml of Na2EDTA solution at a concentration of 0.4 molar, which was prepared with a weight of (14.88) g of it and dissolved in (80) ml of distilled water, then the pH was adjusted to (8) using a solution of NaOH (1) molar, then completed the volume to (100) ml of distilled water, then 20 ml of Tris-HCl solution at a concentration of (1) molar was added to it, which was prepared by weight of 12.1 of it and dissolved in 30 ml of distilled water, then the pH was adjusted to (8) using a solution of HCl (1) molar, then completed the volume to 100 using distilled water, then complete the volume of the extraction solution to 200 ml and then adjust the pH to (8) using a solution of NaOH (1) molar, and sterilize with an autoclave at a temperature of 121°C and a pressure of 1.5 atmospheres for 15 minutes.

2- Washing buffer: Prepare 100 ml of it by dissolving 0.136 g of ammonium acetate in 76 ml of absolute ethanol, and fill the volume to 100 ml with distilled water.

3- Chloroform solution: isoamyl alcohol (1:24): To prepare 100 ml of it, mix 96 ml of chloroform with 4 ml of isoamyl alcohol, and keep it in an airtight, opaque bottle at a temperature of 4 °C.

4- TE solution: This consists of:

- 0,010 molar Tris-HCl
- 0.001 molar Na2 EDTA.

It is prepared at a concentration of 10 mg/ml by dissolving 100 mg of dye powder in 10 ml of distilled water and kept in a sterile vial at a temperature of 4° C until use. 40 microliters are withdrawn from it and mixed with one liter of distilled water to prepare the agarose gel dyeing solution.

The method of isolating DNA from chickpea leaves:

- ❖ Young leaves were taken from each sample, washed with distilled water, dried, then weighed (0.5) g of them and cut and placed in a ceramic mortar and liquid nitrogen was added to them, then they were ground well until the sample became a greenish-white powder.
- ❖ The powder was placed directly in glass tubes and (5) ml of CTAB extraction solution was added to it in a water bath at (65) C. The tubes were incubated in a water bath

with the tubes constantly shaking at a temperature of (65) C for a period of 90 minutes.

- The glass tubes were removed from the water bath and left to come to room temperature, then 4 ml of chloroform solution : isamyl alcohol (1:24) were added to each tube with continuous stirring for 15 minutes.
- The tubes were placed in a refrigerated centrifuge and the mixture was discarded at a speed of 5000 rpm for 15 minutes at a temperature of (4)°C.
- After the expiration time, the upper aqueous layer was lifted by means of a micropipette to another tube and 4 ml of chloroform solution: isoamyl alcohol was added, and centrifuged at 5000 rpm for 15 minutes at a temperature of (4) °C.
- The upper aqueous layer was re-lifted by means of a fine pipette after the end of the centrifugation and placed in new sterile tubes, and a similar amount of cooled ethanol was added to it and mixed by quiet stirring until a white mass appeared representing the DNA strands.
- The DNA strands were withdrawn using a glass rod with a hook end and placed in a tube containing (2) ml of washing solution and left for 20 minutes.
- Then it was lifted by the rod into sterile tubes containing 100-200 microliters of the dissolving solution and by stirring from time to time until the DNA was completely dissolved, and the DNA samples (stock sample) were kept at -20°C for later use.

Determination of the concentration and purity of the extracted DNA:

The Nano drop device was used, which gives direct and quick results for the concentration and purity of DNA on the computer connected to the device, and by uploading only one microliter of DNA to the device, which reads it at wavelengths of 260 nm and 280 nm and then dividing the reading of 260 nm by 280 nm gives the result The ultimate for purity and concentration.

Determination of the molecular sizes of DNA:

Electrophoresis was performed on an agarose gel with the aim of quantifying the molecular sizes of DNA, using the DNA Volumetric Guide (100 bp DNA Ladder) known as the molecular weight.

Solutions used in the migration process:

1- TBE (Tris Borate EDTA) Solution 10X:

This solution consists of:

- 0.89 M Tris-base
- 0.89 molar Boric acid
- 0.02 M EDTA 8pH

To prepare 100 ml of TBE 10X solution, take 10.8 g of Tris-base with 5.5 g of boric acid and dissolve them in 80 ml of distilled water, then add 5 ml of Na2EDTA solution at a concentration of 0.4 M and adjust the pH to 7. 8, and then complete the volume to 100 ml with distilled water and then sterilize with autoclave. At each use it is diluted by adding nine parts distilled water to obtain TBE X 1. Maniatis (Maniatis et al. 2001).

2- Loading buffer with a strength of 10X:

This solution is prepared by dissolving 0.25 g of bromophenol blue dye in 50% glycerol, then add EDTA solution at a concentration of 60 mM (PH = 8) and complete the volume to 100 ml with distilled water. It is kept at 4°C until use.

3- Ethidium bromide dye:

It is prepared at a concentration of 10 mg/ml by dissolving 100 mg of dye powder in 10 ml of distilled water and kept in a sterile vial at a temperature of 4° C until use. 40 microliters are withdrawn from it and mixed with one liter of distilled water to prepare the agarose gel dyeing solution.

Method of preparing acarose gel and the process of DNA electrophoresis [13]

For genomic DNA migration, detection and estimation of its molecular size, agarose gel was prepared at a concentration of 1% by dissolving 1.5 gm of agarose powder in 150 ml 1X TBE and placing it on a magnetic stirrer under continuous heat until the temperature reached 50-60 °C. Or by placing it in a Microwave device for two minutes.

- The special comb for the formation of wells is fixed on one end of a special basin tray with the relay device, and after the gel solution cools down a little, it is poured into the basin, and the pouring is done quietly to avoid the formation of bubbles, and if they are formed, they must be removed using a pipette, then leave the gel to solidify.
- Lift the comb and place the Tray in the electric relay trough containing an appropriate amount of TBE 1X solution to cover the loading pits.
- Migration samples are prepared by mixing 5 µl of the DNA sample with 3 µl of loading buffer using a micropipette.

- The samples are carried in the gel pits, as well as the DNA volumetric guide (DNA Ladder) in a special hole on one side of the gel.
- The relay device is then operated by passing the electric current with a voltage difference of (3) volts / cm. After adjusting the poles, the direction of relaying the samples should be towards the positive pole until the samples arrive before the end, and the process takes 60 minutes.
- After completion, the gel is transferred to a basin containing ethidium bromide dye with a final concentration of 0.5 µg/ml and left for half an hour with continuous stirring.
- Photographing the gel using the E-Graph Gel documentation system, which contains a high-resolution digital camera to photograph the gel with a UV lamp on the bottom, and the captured images are stored directly on the computer.

Estimation the molecular weights

The molecular weights of the DNA bundles were estimated based on the distance they traveled in the gel, which is inversely proportional to their molecular weights and in the presence of standard size indices (Markers). A graph is drawn between the logarithm of the molecular weights of the volumetric DNA bundles on the y-axis, and the logarithm of the distance traveled by each bundle in the gel on the x-axis, and based on this curve the molecular weight of each bundle is estimated.

RAPD interactions:

RAPD reactions were performed according to [12] on DNA samples (7 pure strains and 21 single crosses).

Substances and solutions required for the reaction.

- Premix buffer solution: that was obtained from Bioneer Company.
- Random Primers: Using (38) a primer as shown in Table (9), which was supplied by Operon Technologies, U.S.A.
- DNA template.
- Distilled sterile water.

RAPD technology

- The work was carried out wearing gloves and under a sterile hood, with all solutions kept on ice.

- The concentrations of the studied DNA samples were adjusted by dilution with sterile distilled water to reach the final concentration for conducting RAPD reactions, and it is approximately (50) ng/microliter for each sample.
- The master reaction mixture was prepared by mixing the reaction components in a sterile 0.2 ml Premix tube, as shown in Table (10):
- Then it was placed in the thermocycler on a special program, as follows: One cycle of 4 minutes at a temperature of (94) C for the initial denaturation of the DNA strand, then followed by (40) replication cycles including each cycle of 30 seconds at a temperature of (92) C for the denaturation of the double strand, and 45 seconds at a temperature of (36) C for the binding of the initiator to the DNA strand. Template DNA was 45 seconds at (72) C to elongate the initiator, then a final cycle for 7 minutes at (72) C to complete the elongation phase.
- After the end of the reaction time, the tubes were lifted from the thermopolymer device and 5 microliters were withdrawn from the tubes and loaded with etching the previously prepared agarose gel at a concentration of 1.5%, with the marker loaded on one side.
- Then the samples were deported by running the electrophoresis for 90 minutes.
- The gel was then dyed with ethidium bromide for 30 minutes, stirring with the shaker device, and then exposed to ultraviolet rays on the device. Gel documentation system

Results & Discussion

Evaluating the performance of individual genotypes and hybrids for phenotypic traits

Flowering time (day)

It is clear from the results in Table (1) that the highest time taken to reach the flowering date of the plant was for the father (2), which reached (109.1) days, while the lowest period for the maturity date for the father (5) was (89.2) days, as for the hybrids In Table (2), the hybrid (2 × 6) gave the highest flowering period of (115.60) days, while the hybrid (1 × 5) had the lowest flowering period of (87.56) days, and this indicates the transmission of this trait from the mentioned parents that excelled When observing the results of the general average, we note that the parents had the shortest flowering period and amounted to (102.22) days compared to the average crosses that gave the highest flowering period and reached (104.97) days. This means that the genetic structures that gave the highest duration of access to flowers may give a greater opportunity to form a suitable vegetative cover, and

as a result of the long period of photosynthesis, it leads to storing more materials in the stem and leaves, so the chance of transferring a large amount of nutrients through the grain increases. Thus, it is positively reflected on the final yield, and thus it is possible to take advantage of the father (5) and the hybrid (5×1), which took the shortest flowering period, in order to transfer their good genetic traits to the local cultivated varieties [5].

The plant height

The results of tables (1) and (2) were indicate that the highest plant height for the father (6) that reached (63.6) cm, while the lowest plant height for the father (5) was (56.5) days. As for the hybrid, it gave the hybrid (3×). 7) The highest height of the plant reached (67.06) cm, while the hybrid reached (2×3) with the lowest branches of the plant reached (34.13) cm, and this indicates that the parents mentioned have transferred this trait that they excelled with to their hybrids. The highest plant height was (59.2) cm compared to the average hybrids that gave the lowest plant height (51.72) cm. Thus, the father (6) and the hybrid (7 × 3) are the shortest in height and can be benefited from, because the short and strong stems are a necessary factor to avoid The occurrence of sluggishness, and then this negatively affects the final yield, which indicates the response of the plants of the first generation to the decrease in plant height, and this agrees with [5]. Also, this is agreed with [8].

Number of pods/plant

It is noticed from the results of the trait of the number of horns shown in Table (1) that the father (5) was significantly superior and amounted to (112) horns, while the father (3) gave the lowest value for the same trait, which amounted to (3) horns. As for the camels in Table (2), it was the hybrid (1 x 5) gave significant superiority with an average of (97.10) pods, while the hybrid (1 x 6) gave the lowest averages and reached (61.73) pods. The average of the parents (83.17) pods for this trait indicates that the response of some parents and their hybrids to the increase in this trait is due to the response of some of these parents and their hybrids in the characteristic of the ratio of knots, which affected the increase in the number of pods / plant and this positively affects the increase in the final yield, for Achieving high productivity, as it is necessary to ensure the use of genotypes that have a genetic potential that gives a high rate in this trait, which is one of the important components of the crop and that qualifies it for high production [13-14-17-18].

The number of seeds in the pod

It appears from the results in Tables (1) and (2) that the father (1) was significantly superior in the number of seeds/pod and reached (85.28) seeds, and differed from the father (5) and

gave the lowest value for this trait, which amounted to (52.16) seeds. As for the hybrids, it was The hybrid (1 × 5) gave significant superiority, with an average of (79.16) seeds, while the hybrid (4 × 7) gave the lowest average and reached (34.50) seeds. Parents average (62.51) seeds, and this trait can be used to improve the yield, as the genotypes that have a sufficient amount of dry matter during the stage of formation and development of the ears may increase the percentage of fertile florets and reduce the percentage of sterile and aborted florets, which leads to an increase in the number of Seeds in one pod, which reflected positively on the final yield, and these superior genetic structures can be used in cross-breeding programs to improve grain yield or benefit from them in the future and register them as new varieties, and this is consistent with what was found by [5, 14].

Weight of 100 seeds (gm)

The characteristic of the average seed weight (gm) is one of the important components of the yield and is thus an effective indicator of the efficiency of the transfer and representation of manufactured materials from the source to the downstream Sink in the storage sites of the seed, from the results contained in Table (1) we note the superiority of the father (4) Significantly, it amounted to (43.69) g, while father (4) gave the lowest value for this trait amounted to (31.89) g. As for the crosses in Table (2), the hybrid (1 × 5) gave a significant superiority with an average of (68.80) g, while the hybrid gave (4×5) was the lowest of the averages and amounted to (43.61) g. When comparing the average of the parents with the average of the crosses, it was distinguished by the highest value of the hybrids, which amounted to (53.43) gm, while in the parents it reached (37.12) g. The difference between the genotypes may be due to the difference in the number of seeds/ Pod as a result of the different lengths of the pods in plants, which is determined by the amount of photosynthesis products from the source to the estuary, and this is determined by the interaction of different genotypes with the elements of the available environment, and this is consistent with what [8-18] found.

Seed yield (kg/ha-1)

The trait of grain yield is considered a final outcome in most of the physiological and phenotypic traits of plants, as the increase of this trait and its components is an important achievement for the plant breeder who seeks it in most plant breeding programs to obtain a variety with good production capacity under different conditions, it is noted from tables (4) and (5) that the father (5) produced the highest average amounted to (1422.6) kg/ha-1, while father (4) produced the lowest average for this trait reached (186.7) kg/ha-1, while the

hybrid (1×5) showed the highest An average of (1369.43) kg/ha-1, while the hybrid (4×7) gave a lower yield of (929.69) kg/ha-1, and the average of the hybrid was significantly superior to the average of the parents, which was (848.11) kg/ha-1. The average of the parents is (1104.556) kg/ha-1, and by evaluating the arithmetic averages of the parents and the crosses, and that this superiority that came in the seed yield came as a result of their superiority in the components of the yield, which was positively reflected on the final yield. The dryness of the seeds and the increase of photosynthesis during the maturity stage, which reflected positively on the final yield and this agreed with [5]. From the foregoing, it is noted that the genotype (1) and (5) and the hybrid (1×5) are superior in most of the studied traits, which can be evaluated in more than one year and site to benefit from and adopt them in agriculture as they are among the promising inputs. The genetically divergent genotypes are among the important factors for obtaining highly productive hybrids in the characteristics of the yield and its components. If the genetic divergence increases, the genetic differences between the parents increase, and this works to collect the good and desirable genes in the resulting hybrids, and this also increases the chances of an increase in the general and private ability to combine [13].

Predicting the performance of parents and individual crosses by DNA indicators

Results of RAPD reactions

The results of the RABD primers used in this study varied, which showed different bundles and are shown in Table (3), as thirteen primers did not show any replication products (DNA bundles) on the agarose gel, namely: OPC-13, OPC-16, OPC-20 and OPC -20, OPC-20, OPC-13, OPC-08, OPC-07, OPC-04, OPC-20, OPC-01, OPC-06, and OPC-14, which represented 35,01% of the total number of prefixes used in the study, while the remaining prefixes were found to have complementary sequences. On the DNA of the studied genotypes, it represented 66.79% of the total primers, and the total packages produced by these primers were (150) packages, with an average of approximately (6) packages per initiator, and the number of packages ranged between (1) packages for the initiator OPA-09 and (9) Bundles for OPC-08, OPC-18 and OPC-08 primers, all primers showed different bundles except for the primer OPC-18, which showed only one main bundle for all genotypes with a molecular size of (800) bp, and an efficiency of (0.671%). The total of the disparate bundles in the prefixes that showed different bundles was (133) bundles, which represented (89.261%) of the total resulting bundles, with an average of approximately (5) bundles for the initiator, and it ranged between (3) bundles in the prefixes OPC-09, OPC-02 and OPC-08 And OPC-17 and (9)

packages for the OPC-08 and OPC-08 starters, and the overall results above are good and similar to the results obtained by some studies, and superior to the results of other studies, including: Drinic et al. (2012) and Tomkowiak et al. (2013),

- OPC-01 initiator: The results of this initiator showed the presence of (6) bundles whose molecular sizes were limited between (600-1500) bp, and ranged from (1-5) genotype bundles. A percentage of (100%), and accordingly, the efficiency of this initiator reached (4.02%), while its differentiating ability reached (4.51%), and two genetic structures were distinguished: the father (3), who was distinguished by an absent bundle whose molecular size reached (600) bp and the father (4) which was distinguished by a visible band with a molecular weight of (1500) bp.
- OPC-02 initiator: This initiator produced (3) packets whose molecular size ranged between (700-1145) bp, and with an average of (1-3) packets for the genotype. The number of differentiated bundles produced by this initiator was (3) bundles, thus forming a percentage (100%) of the number of bundles produced, and this initiator recorded an efficiency of (2.013%) and a discriminating ability of (2.256%), and the hybrid (1x6) was distinguished by a visible bundle. With a molecular size of (700) bp.
- Initiator OPC-03: The number of bundles for this initiator was (8) bundles, and their molecular sizes were limited between (400-1580) bp, and at a rate of (1-5) bundles for genotype, and (7) bundles showed a variation of (87, 5%) of the number of packages. Accordingly, the efficiency of this initiator reached (5,369) and its discriminating ability reached (5.263%), and the father (3) was distinguished by a visible package with a molecular size of (800) bp.
- OPC-01 initiator: This initiator achieved (5) bundles whose molecular sizes ranged between (775-1560) bp, and the number of bundles ranged between (1-5) genotype bundles, and the number of different bundles in the initiator reached (4) bundles represented (80%) of the number of bundles, and accordingly, the efficiency of this initiator reached (3,356%), while its discriminating ability reached (3,008%). bp for the two packets.
- OPC-07 initiator: The studied DNA of the genotypes was doubled with this initiator within PCR reactions, and this initiator showed (8) bands varying in molecular size that ranged between (600-1500) bp, and at a rate of (2-6) bundles, The number of dissimilar packets in this initiator was (8) packets (100%), and as the ratio of the total number of packets shown by this initiator to the total produced by all initiators, it has an efficiency of (5.369%), and depending on the ratio between the number of

dissimilar packets of this initiator To the total number of different bands produced by all the primers, the discrimination ability of this primer was (6.015%), and father (6) was distinguished by an absent band with a molecular size of (500) bp, and the hybrid (2x4) was distinguished by a visible band of molecular size (1500) bp, and these two bundles are the markers for these two genotypes.

- OPC-09 initiator: This initiator produced (3) packets whose molecular size ranged between (500-1000) bp, and at a rate of (1-3) bundles for genotype, and the number of divergent bundles produced by this initiator was (3) bundles, i.e. a percentage of (100%), while the efficiency of this initiator was (2,013%) and with a discriminating ability (2,256%), and this initiator produced a characteristic absent packet of marker for the father (3) with a molecular size (1000) bp as well as produced an apparent packet with a molecular size of (500) bp characterized Hybrid (1x5).
- Initiator OPC-11: The number of bundles shown by this initiator was (5) bundles, their molecular sizes were limited between (600-1760) bp, and at a rate of (1-3) bundles for genotype, and the number of varying bundles reached (4) bundles formed The percentage of (80%) of the number of packages, and the efficiency of this initiator was (3,356%), while the discriminating ability was (3,008%). This initiator showed a distinct hybrid visible band (1x5) with a molecular size of 600bp.
- OPC-19 initiator: The outputs of this initiator were represented by number of (4) bundles, whose molecular sizes ranged between (630-1340) bp, and they ranged between (1-4) bundles for genetic structure. The number of the different bundles was (4), ie (100%) of the number of bundles, and thus its efficiency was (2.685%) and its discriminatory ability was (3.008%).
- Initiator OPC-07: This initiator achieved a number of packets that reached (7), their molecular sizes were limited between (400-1690) bp, and the number of bundles varied between genotypes ranging from (2-6) bundles, and the number of different bundles that it achieved reached (6) Bands represented a percentage (85.714%) of the number of bands, and the efficiency of this initiator was (4.698%), while its discriminating ability reached (4.511%), and the father (3) was distinguished by an absent band with a molecular size of (800) bp.
- The initiator OPC-08: The number of packets recorded by this initiator was (9) packets whose molecular sizes ranged between (400-1500) bp, and at a rate of (2-7) bundles for genotype, and the number of different bundles in the initiator was (9) packets with a percentage of (100%) of the number of packages. Accordingly, the

efficiency of this initiator reached (6.04%), while its discriminating ability reached (6.767%). This primer was distinguished for parents (3) and (6) with apparent bands with molecular size (450 and 400) bp for the parents, respectively.

- Initiator OPC-15: The number of bundles achieved by this initiator was (5) bundles, their molecular sizes ranged between (300-1530) bp, and the number of bundles for genotypes ranged between (1-4) bundles, and the number of differentiated bundles achieved was (4)) Packages constituted (80%) of the number of packages. The efficiency of this initiator was (3,356%), while the discriminating ability was (3,008%). Father (6) was distinguished in this initiator by an apparent band with a molecular size of (1530) bp.
- Initiator OPC-05: This initiator achieved a number of (7) bundles whose molecular sizes were limited between (280-1200) bp, and were limited to (3-7) bundles for genotype, and the number of different bundles in the initiator reached (6) A package represented (85.714%) of the number of packages, and thus its efficiency reached (4.698%), while its discriminating ability reached (4.511%).
- Initiator OPC-08: The results of this initiator showed the presence of a number of (4) bundles whose molecular size ranged between (375-1580) bp, and the number of bundles ranged between (1-4) bundles for genotype, and the number of differentiated bundles In the initiator, it reached (3) bundles forming (75%), and the efficiency of this initiator reached (2.685%), while its discriminating ability reached (2,256%), and the father (6) was distinguished by an absent distinct bundle, whose molecular size was (370) bp. .
- OPC-18 initiator: This initiator produced (9) bands whose molecular size was limited between (375-1200) bp and at a rate of (4-8) bands for the genotype. The number of different packages in this initiator was (7) packages, which represented (77.777%) of the number of packages, and accordingly, the initiator's efficiency reached (6.04%) and the discriminating ability (5.263%).
- Initiator OPC-07: The number of packets in this initiator was (5) bundles, their molecular sizes ranged between (400-1000) bp and at a rate of (2-4) bundles for genotype, and the number of divergent bundles in it was (5) bundles, or by (100) %) of the number of packets. The efficiency of this initiator was (3,356%), while the discriminatory ability was (3,759%). The results of this initiator indicate that parents (3) and (6) have a visible band of molecular size (1000 and 900) bp for the parents, respectively.

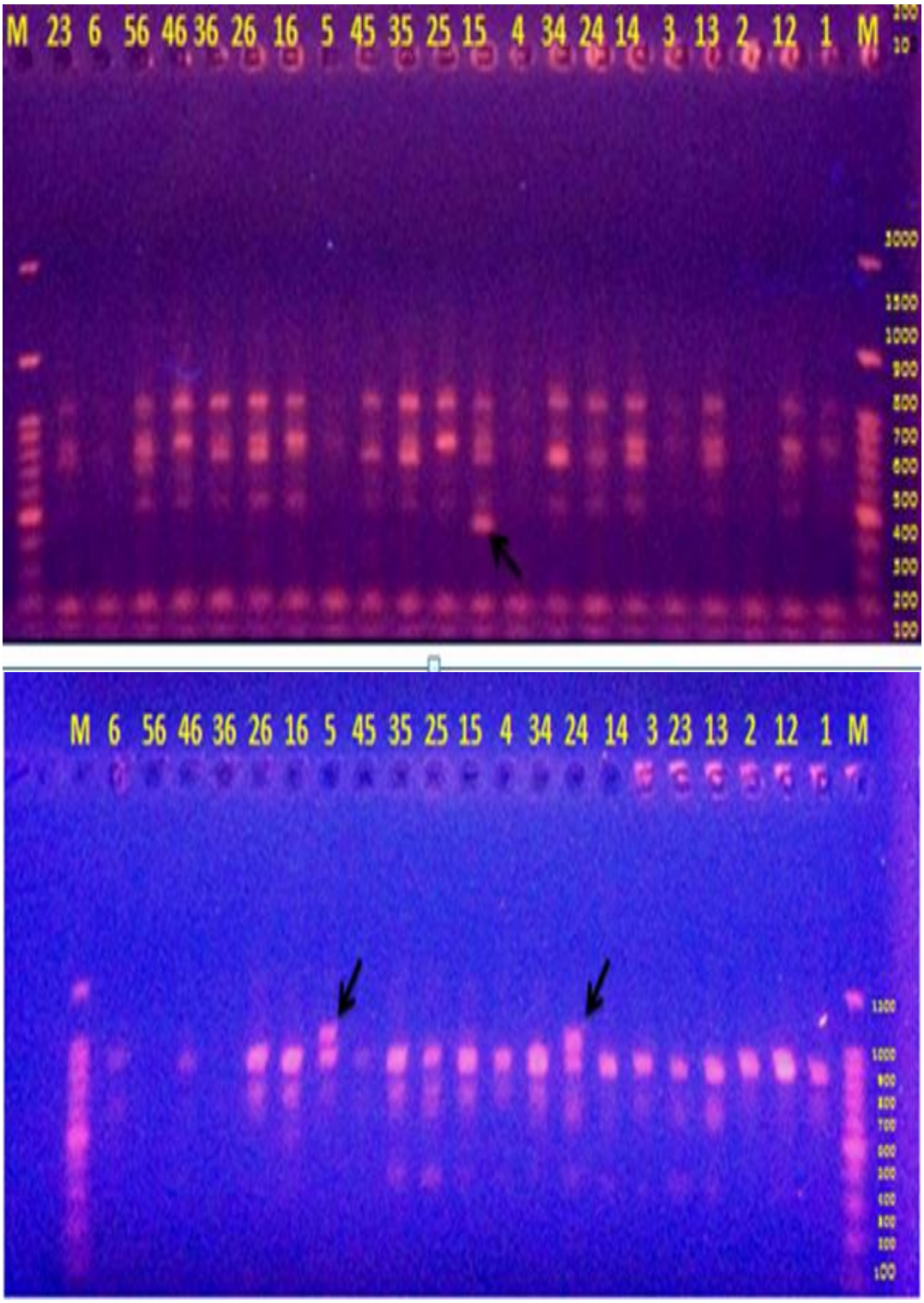
- OPC-10 initiator: The results of this initiator show that the number of bundles in it reached (6) bundles, the molecular sizes of which ranged between (500-1465) bp, and the number of bundles ranged between (1-6) bundles for genotype, and all the resulting bundles were dissimilar forming a ratio (100%), and accordingly, the efficiency of this initiator was (4.027%), while its discriminating ability was (4.511%). for two straight hybrids.
- OPC-12 initiator: This initiator showed a number of (5) bundles whose molecular size was limited to (600-1110) bp, and the number of bundles ranged between (1-4) bundles for genotype, and the number of different bundles in this initiator (5) Packages formed accordingly (100%) of the number of packages, and the efficiency of this initiator reached (3,356%), while its discriminating ability reached (3,759%).
- OPC-01 initiator: This initiator recorded a number of (7) bundles whose molecular sizes were limited between (500-1000) bp, and at a rate of (3-5) genotype bundles, and the number of different bundles in this initiator was (6) formed bundles. Thus, a proportion of (85.714%) of the number of bundles, and its efficiency reached (4.698%), while its discriminating ability reached (4.511%), and this initiator recorded that the father (3) had two bundles, the first absent and the other visible, with molecular sizes of (1000 and 900) bp for the two bundles, respectively.
- Initiator OPC-06: The number of packets achieved by this initiator was (5) bundles whose molecular sizes ranged between (800-1720) bp, and the average number of bundles was (1-4) for genotypes, and the number of different bundles in this initiator was (4) Bundles represented (80%) of the number of bundles, and the efficiency of this initiator reached (3.356%), while its discriminating ability reached (3.008%). Father (2) was characterized by a visible band with a molecular size of (1500) bp.
- Initiator OPC-17: The results of this initiator showed that the number of bundles in it was (4), their molecular sizes ranged between (900-1780) bp and the number of bundles ranged between (1-4) bundles for the genotype, and the number of different bundles in this initiator (3) Packages at a rate of (75%) of the number of packages, so its efficiency reached (2.685%) and its discriminating ability reached (2.256%).
- Initiator OPC-08: This initiator is one of the primers that produced the highest number of bundles of (9) whose molecular sizes ranged between (270-1310) bp and at a rate of (4-8) bundles for genotype. (100%), and therefore the efficiency of this

initiator reached (6.04), while its discriminating ability reached (6,767), and through the results it is also noted that the hybrid (1x2) has an absent band with a molecular size of (1310) bp.

- ❖ OPC-13 initiator: This initiator achieved a number of (7) bundles whose molecular sizes ranged between (360-1200) bp, and the number of bundles ranged between (2-6) bundles for genetic structure, and the number of different bundles in this initiator was (6) Packages represented (85.714%) of the number of packages, and it achieved an efficiency of (4.698%), while it achieved a discriminating ability of (4.511%).
- ❖ Initiator OPC-09: This primer produced (8) bundles whose molecular size was limited between (480-1500) bp, and with a rate of (1-7) bundles of genotype. All bundles produced by this initiator were different, constituting (100%) of the number of bundles produced, and the efficiency of this initiator reached (5,369%), while its discriminating ability reached (6.015%), and the hybrid (3x5) was characterized by an absent bundle with a molecular size of (700)) bp.
- ❖ OPC-10 initiator: This initiator was among the primers that achieved the largest number of bundles (9), with molecular sizes ranging between (200-1590) bp, and the number of bundles for genotypes ranging between (1-8) bundles, and reached The number of different packages achieved by (7) packages constituted (77.777%) of the number of packages. Accordingly, the efficiency of this initiator was (6.04%), while the discriminating ability was (5.263%), and the hybrid (1x6) was distinguished in this initiator by a visible band with a molecular size of (1590bp).

The results of these prefixes showed clear and reliable packets, as they excluded faint packets, which may result when a slight defect in the interaction conditions occurs, or it may be a real packet, but it has been exceeded, which cannot be read. This type of packets is present in most of the results of previous studies. , the sources indicate that analyzing the results of all DNA indicators, including RAPD indicators, depends on the presence or absence of bundles, as well as on the number of bundles and their molecular sizes (Smith and Smith, 1992). The appearance of any bundle on the agarose gel means that the initiator sequence found a complementary sequence to it. On the genomic DNA of the studied model, and that the absence of that bundle in the genome of another model is nothing but a lack of the linking site of the initiator, and on this basis the disparities are built between individuals or groups, and so on. In the presence of those sites that will later be represented by the bands on the agarose gel, the methods of molecular discrimination in the RAPD indicators on the

appearance of distinctive bands may emerge the importance of this case when the absence of the main band from one of the hybrids or the presence of a distinctive band appeared Al-Sakmani (2017) indicated that the use of several primers leads to the detection of discrepancies between the studied genotypes, and that the absence of bundles in genotypes and their presence in others. It explains this contrast. Some primers had the ability to distinguish more than one genotype, such as the initiator OPC-5, which showed distinct bands with the rest of the hybrids, as well as the initiator OPP-01 showed distinct bands with the ability to distinguish two hybrids (15,27). Such primers are the focus of researchers' attention About DNA Fingerprinting as it shortens the efforts and capabilities to reach their goals with the least number of interactions, and this opens the horizon for the future not only to find distinctive packages for other strains, but also to link such pieces with other field or analytical characteristics to facilitate the task of plant breeders (Al-Qaisi, 2013), agreed The results of this study with many researchers in this field. In the study of Al-Ghamdi (2009) on eight parents and their individual crosses of the bean plant, four primers were used in the indicators of the RAPD.


M 23 6 56 46 36 26 16 5 45 35 25 15 4 34 24 14 3 13 2 12 1 M
M 6 56 46 36 26 16 5 45 35 25 15 4 34 24 14 3 23 13 2 12 1 M

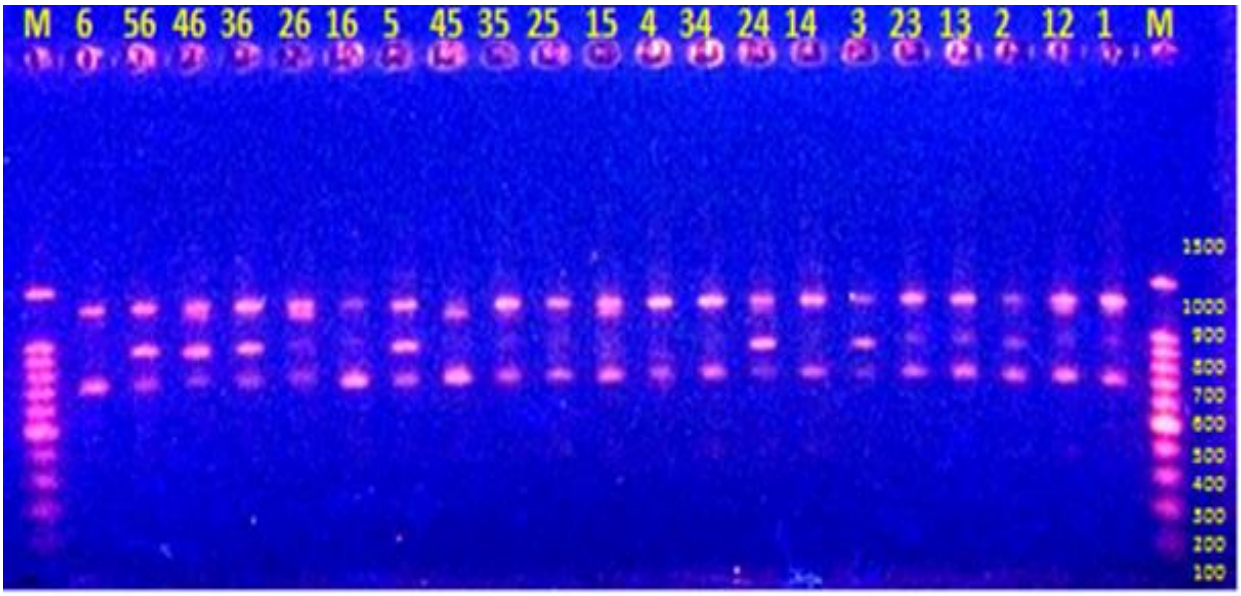


Figure (1): Replications of primers with DNA of individual hybrids of the first generation and their six parents and the stage on agarose gel at a concentration of 1.5% with volume index M

Genetic dimension

Based on the values of the genetic dimension among the genotypes, a genetic relationship was found between them and in the form of Clusters Figure (1), where the genotypes were divided into four main groups: the first main group included the strain (A134), while the second main group included the strain (A133) and it included The third main group, strain (H130), while the fourth major group was divided into two secondary groups, the first included strain (H129), while the second included strains (H126) and (H118). This means that the genotypes that comprise one group are close in their phenotypic performance of chemical traits and benefit the breeder by entering those genotypes that are divergent in their performance into the cross-breeding programmes. The best and which were stable in the expression of their genes for these traits can also be selected to ensure their superiority under the study factors (Al-Maliki, 2017). It is concluded from the above that it is possible to use the RABD indicators in dividing the chickpea plant strains into different groups and estimating the genetic dimension between them, which is directly related to the performance rate of the hybrids, the strength of the hybrid and the special ability to combine, and it is efficient in predicting the performance of hybrids that give high productivity advantages. These results are consistent with the findings of Al-Ghamdi (2009) and Al-Sakmani (2017).

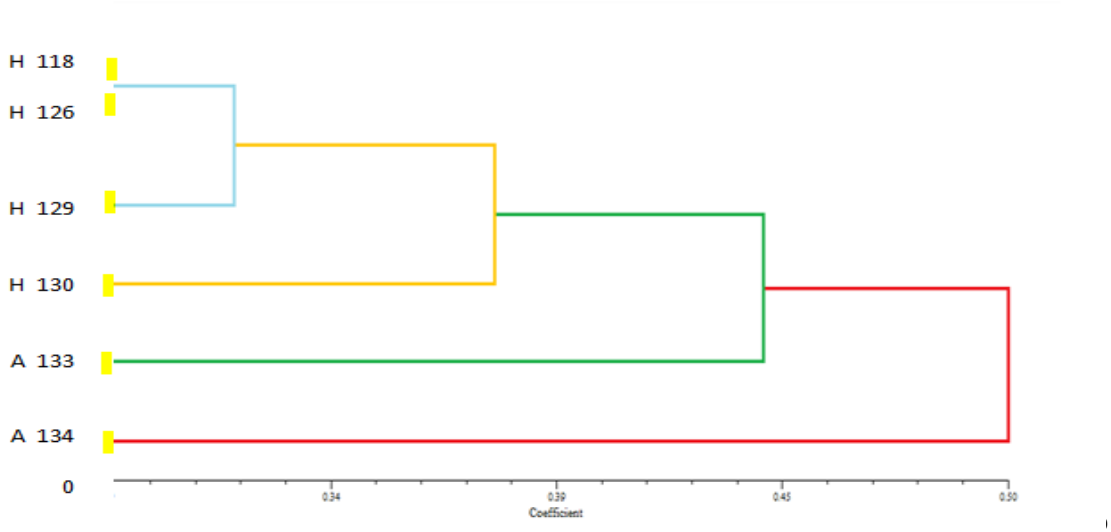


Figure (2) also shows the values of the genetic dimension among individual hybrids. The relationship between individual hybrids was determined in the form of a tree that distributed them into groups based on the values of the genetic dimension between them. The groups were as follows:

- The first main group: This group contained only two hybrids (5 x 6) and (26 x), and they shared one father, the father (6). Referring to the results of table (48), it is noted that the father (6)'s contribution to the genetic material of these two hybrids was greater than the contribution of the other father.
- The second main group: This group was divided into two subgroups:
- Secondary group I: It included the hybrid (3x6), whose parents had the highest genetic dimension between them.
- Secondary group: This group was divided into two branches; the first included the hybrids (3x4) and (3x5). These two hybrids shared the father (3), who had a clear illiterate effect on them, while the second branch included the two hybrids (2x3) and (2x4), who shared By the father (2) who imposed the influence of his cytoplasmic inheritance as a mother on the two hybrids.
- The third main group: This group was divided into two subgroups:
- The first secondary group: This group included the hybrid (4x6) only.
- Second secondary group: This group was divided into two branches, the first included the two hybrids (1x3) and (1x6), which shared the father (1), who imposed his illiterate influence on them, and the second branch was divided into smaller

branches, the first of which included the hybrid (2x5) and the second the hybrid (4x5).) While the third included camels (1x5), (1x4) and (1x2), and these three crosses shared the father (1), whose illiterate influence on them was clear.

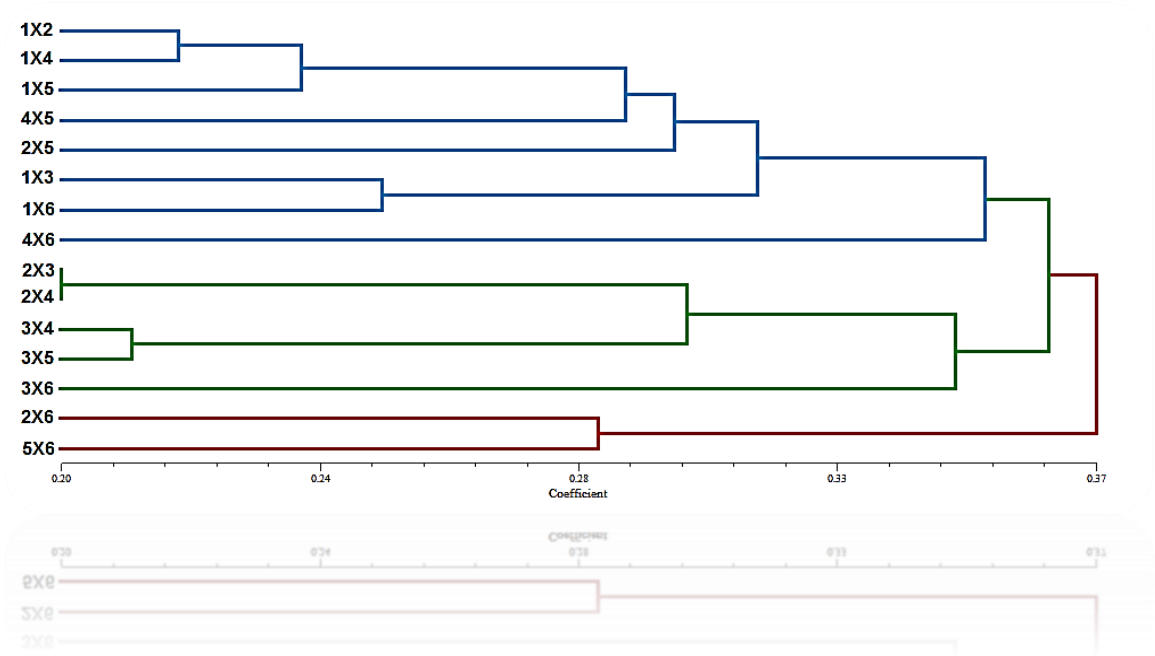


Figure (3): represents the genetic relationships and groups of individual crosses according to RAPD indicators

Table (1): The averages of the fathers for the studied traits

Proprieties / **fathers**	flower time (day)	plant height/cm	Number of pods/plant	Number of seeds/pod	Weight of 100 seeds (gm)	Seed yield (kg.ha-1
1	91.3	61.3	63	85.28	35.76	1210.5
2	92. 3	55.8	91	62.46	41.81	913.6
3	103.2	57	61	52.16	33.78	720.8
4	105.3	61	101	46.38	31.89	186.7
5	89. 2	56.5	112	54.93	43.69	1422.6
6	109.1	63.6	63.6	73.89	35.79	634.5
Mean	102.22	59.2	81.93	62.51	37.12	848.11

Table (2): averages of hybrids for the studied traits

Proprieties / Hybrid	flower time (day)	plant height/cm	Number of pods/plant	Number of seeds/pod	Weight of 100 seeds (gm)	Seed yield (kg.ha-1
1×2	105.30	56.03	78.53	77.53	45.71	1290.35
1×3	113.93	56.26	88.26	64.53	56.84	1048.22
1×4	104.60	62.10	87.06	58.40	45.73	937.16
1×5	87.56	45.06	97.10	79.16	68.80	1369.43
1×6	109.20	52.73	61.73	74.66	56.55	1192.81
2×3	94.23	34.13	86.13	45.20	56.72	1024.84
2×4	92.51	54.40	91.40	57.30	45.56	1089.28
2×5	113.20	45.90	85.90	77.03	45.60	1283.43
2×6	115.60	65.03	78.03	66.76	57.71	816.03
3×4	114.46	56.76	87.76	66.80	56.79	1285.02
3×5	89.06	45.73	84.73	67.80	61.76	1018.96
3×6	106.66	43.43	91.43	75.26	43.69	1104.10
4×5	115.06	45.36	76.36	59.20	43.61	989.26
4×6	109.30	61.53	65.83	78.93	61.73	1079.18
5×6	103.93	51.36	87.36	77.16	54.71	1040.27
Mean	104.97	51.72	83.17	68.38	53.43	1104.556

Table (3): shows the outputs of the primers from the DNA bundles, their efficiency ratios and their discriminating ability

r	Total number of packets generated	Molecular size range of bp. packets	output packet rate	The number of dissimilar packages	Percentage of dissimilar packages %	The number of identical packages	Distinguished genotype			Efficiency %	discrim abil
							Premium structure symbol	Molecular size of characteristic bundles bp	Excellence type		
	6	1500-600	5-1	6	100	0	3 4	600 1500	Absent Present	4,027	4,5

3	1145-700	3-1	3	100	0	6X1	700	Present	2,013	2,
8	1580-400	5-1	7	87,5	1	3	800	Present	5,369	5,
5	1560-775	5-1	4	80	1	5X3 5X3	770 800	Present Present	3,356	3,
8	1500-600	6-2	8	100	0	4X2 6	1500 500	Present Absent	5,369	6,
5	1000-400	4-2	5	100	0	3 6	1000 900	Present Present	3,356	3,
5	1760-600	3-1	4	80	1	5X1	600	Present	3,356	3,
3	1000-500	3-1	3	100	0	3 5X1	1000 500	Absent Present	2,013	2,
7	1690-400	6-2	6	85,714	1	3	800	Absent	4,698	4,
9	1500-400	7-2	9	100	0	3 6	450 400	Present Present	6,040	6,
5	1530-300	4-1	4	80	1	6	1530	Present	3,356	3,
7	1200-280	7-3	6	85,714	1	--	--	--	4,698	4,
4	1580-375	4-1	3	75	1	6	370	Absent	2,685	2,
9	1200-375	8-4	7	77,777	2	--	--	--	6,040	5,
4	1340-630	4-1	4	100	0	--	--	--	2,685	3,
6	1465-500	6-1	6	100	0	5X4 6X1	660 1200	Absent Absent	4,027	4,
1	800	1	0	0	1	--	--	--	0,671	0,
5	1110-600	4-1	5	100	0	--	--	--	3,356	3,
7	1000-500	5-3	6	85,714	1	3 3	1000 900	Absent Present	4,698	4,
5	1720-800	4-1	4	80	1	2	1500	Present	3,356	3,
4	1780-900	4-1	3	75	1	--	--	--	2,685	2,
9	1310-270	8-4	9	100	0	2X1	1310	Absent	6,040	6,
7	1200-360	6-2	6	85,714	1	--	--	--	4,698	4,
8	1500-480	7-1	8	100	0	5X3	700	Absent	5,369	6,
9	1590-200	8-1	7	77,777	2	6X1	1590	Present	6,040	5,

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