Ann. For. Res. 65(1): 6342-6359, 2022
ISSN: 18448135, 20652445

ANNALS OF FOREST RESEARCH
DOI: 10.5281/zenodo.7410202

EVALUATION PERFORMANCE AND PHENOTYPIC INDICES OF PARENTS AND THEIR INDIVIDUAL CROSSES AND MOLECULAR CORRELATION GENETIC ANALYSIS BETWEEN RAPD-PCR MARKERS ON THE QUANTITATIVE TRAITS OF PEANUT (*ARACHIS HYPOGAEA* L.)

Saifuldeen Ahmed Hasan[1], Raeed Mejbel Abdullah[2], Mohammed Bustan Hanoon[1] and Muntasser Khudhur Sahi[1]

[1]Shatrah Technical institute, Southern Technical University, Iraq.

[2]Kirkuk Health and Medical Techniques College, Northern Technical University, Iraq.

Emails: drsaif.ahmed@stu.edu.iq , raed.m.abdullah@ntu.edu.iq , Mohammed.hanoon@stu.edu.iq , Muntasir.sahi@stu.edu.iq

Abstract

Seven Peanut genotypes were used, obtained from ICARDA of Dry Cultivation, the parent planted their reverse hybrids in a farmer's field in Dhi-Qar Governorate, using a Randomized Complete Block Design (RCBD) with three replicates, for a molecular study, used two indicators based on PCR technique, namely the RAPD indicators and the RE-RAPD indicator, by cutting enzymes and custom prefixes from the RAPD marker, to determine the correlative genetic relationship between the genotypes of the quantitative traits (Maturity date, number of branches. plant, leaf area, number of pods. Plant, number of seeds. pod, seed weight and seed yield). The results indicated that RAPD indicators could be used, evaluating the genotypes of the Peanut plant, to groups and estimate the genetic distance between them, which was directly related to the molecular genetics and phenotypic genetic distances, the effect of the special unite ability and the strength of the cross between the average parents and the strength of the hybrid on the best parents and the average traits, parent 4 showed the greatest hereditary distance while parent 3 showed the least hereditary distance, based on the results of the phenotypic and RAPD indicators. The RE-RAPD indicators were also efficient in identifying 25 genetic mutations, as these mutations are considered a diagnostic genetic fingerprint of most parents and an indication of the presence of specific sites, especially of parents, in the genome of the parents through the use of 8 primers.

Keywords: molecular genetics, correlation, Peanut.

Introduction

The Peanut (*Arachis Hypogaea* L.) is an important crop, wintry annual plant grown in temperate regions of the world, fairly cold tolerant, cultivation has expanded outside the regions of its origin into Europe and Asia (Sardana et al., 2007). The plants with high nutritional value and important to humans, an important part of food, the main sources of plant protein, reflected on the increase of the global area planted, the quantity of cultivated production in Iraq for the year 2018 was about 518.5 kg. hectare.

The concept of phenotypic indicators, indicators that can be seen with the naked eye, one of the easiest and oldest indicators, considered the basis of indicators, which has been relied upon since the scientist Mendel discovered the basics of genetic until now, these indicators are considered the basis for studying the genetic variation of plant (Hedrick, 2005). Phenotypic indicators are one of

the most important methods of studying the genetic relations between a genotype groups (Raeed and Saifuldeen, 2020) . There are many studies that have been conducted and have demonstrated the ability of phenotypic indicators to show the genetic differences between different varieties or genotypes, however, the determinants that negatively affect the results of those indicators, including environmental, physiological, researchers have trend towards more stable indicators that are less affected by environmental influences (Li et al., 2009).

Selection including molecular markers is to aid phenotypic selection, makes more effective, it was less expensive compared to traditional plant breeding methods (Sabouh et al., 2010), the use of molecular indicators in plant and animal husbandry has been a new field of agriculture, called Molecular breeding (Al-Skmani, 2017).

The development of molecular markers has enabled researchers to overcome all obstacles, it has provided many methods that guarantee accuracy, speed, and shortening effort and time, to pick the best, during early recognition of better genetic performance, the discovery of PCR technology in the past two decades (Raeed and Saifuldeen , 2020) . Many researchers have helped to devise various indicators based on this technology, contributed greatly to the evaluation of hereditary origins, species identification through genetic fingerprint, as well as identifying the genes responsible for many important traits in plant breeding, the important indicators in breeding programs were RAPD PCR-RFLP, AFLP, ISSR, SSR, CAPS and SCAR. RAPD indicators have been used for this purpose, to be surpassed in terms of ease and accuracy, the ability to detect the widest area of the plant genome and lower cost compared to other indicators (Saifuldeen and Raeed , 2020) .

Materials and Methods

The Peanut genotypes were obtained from ICARDA and assigned codes 1-7**,** and 21 individual crosses were the result of these parents, the genotypes of (28) combinations (seven parents and twenty-one single hybrids) were cultivated in a farmer's field in Dhi-Qar Governorate on 25/4/2020. Parents and hybrids were planted with lines, 15 seeds were planted for each line (representing an experimental unit) of 4 m length, each distance between one seed is 0.2 m, and the distance between one line is 0.75 m, according to the Randomized Complete Block Design (RCBD) with three replicates, The block included 28 experimental units, parents and hybrids were distributed randomly, the experiment was irrigated by flow irrigation, conducted plowing, leveling and crop servicing operations according to the followed recommendations. Chemical fertilizer NPK was added in the amount of 400 kg ha after tillage, quantitative traits related to growth and yield characteristics were taken (Al-Jabouri, 2016). Samples were collected from the plants two months after the date of planting from the parents and the hybrids, took 5-6 young leaves from the growing top, placed in special bags marked, was taken to the lab directly for the DNA isolation process. The analyzes, measurement, concentration and purity of DNA were performed in the molecular laboratory of the Faculty of Science at Al-Mustansiriya University.

(RAPD-PCR) reactions:

1.5 g of aerosol powder was dissolved in 100 ml of TAE1X, using a heat source, then the solution is cooled down and poured in special places to prepare an aquarose gel at a concentration of 1.5%, 5 microliters of the RAPD-PCR product were taken for each sample, loaded neatly into the gel pits, as well as the volumetric guide Marker section 100bp-3000bp in a special hole on one side of the gel. The relay is then switched on by passing the electric current with a voltage difference of 3 volts. cm, and after adjusting the electrodes. The direction of the samples to be carried forward should be towards the anode, to the point where samples arrive before the end and the process takes 1.5-2.5 hours.

RE-RAPD reactions:

2 g of agarose powder was dissolved in (100) ml of TAE 1X, using a heat source (microwave), then the solution was cooled and poured in the same way as the previous method in order to prepare a 2% agarose gel, 7 microliters of the RFLP-PCR product were taken for each sample, loaded precisely into the gel pits and the Marker Volumetric Guide (100bp-3000bp) in a special hole on one side of the gel, the process takes 1.5-2.5 hours, the relay was operated by passing the electric current at a voltage difference of 3 volts. cm to the point where the samples arrive before the end, after completion of the migration for transection and unbroken genomes as well as RAPD-PCR and RFLP-PCR reactions. The gel was transferred to a bath containing the ethidium bromide dye to a final concentration of 0.5 g. ml, leave for half an hour with constant stirring, the gel was examined by exposing it to a source of ultraviolet rays on a UV-transilluminator. The jelly was photographed using a high-resolution digital camera, and the images were saved in a computer (Zahra et al. , 2019) .

RAPD-PCR reaction procedure:

The RAPD reactions were performed according to Williams et al. (1990) on samples of the seven parents of Peanut plants using 28 primers used on the parents under study.

First: The solutions and materials used and the supplied company:

1. Green Master Mix (reaction mixture) supplied by promega, U.S.A.
2. The primers were supplied from Operon Technologies, U.S.A.
3. Nuclease-free deionized distilled water supplied by Promega, U.S.A.

Second: the method of work:

1. The DNA concentration in all studied samples was adjusted by dilution with TE solution to obtain the required concentration, for RAPD reactions, it was approximately (50) ng / µL per sample.
2. The main reaction mixture, Rishti Master, was prepared, done by mixing the reaction components in a sterile 2 ml Eppendroffe tube, the mixture was centrifuged in a Microfuge for 2-6 seconds to complete mixing of the reaction components, takes into account that working inside the Hood should be sterile, wear gloves and put the tubes inside ice.
3. The RAPD-PCR reaction program is applied after the reaction time has expired, the tubes are removed from the thermoplastic device and kept in a freeze, 5 Microliters are withdrawn from the tubes, the mixture was loaded onto a pre-prepared acarose gel at a concentration of 1.5%

with the Marker volumetric, then the samples are migrated onto an agarose gel, the gel was dyed by immersing it with ethidium bromide dye for one hour while stirring, and exposed to the UV source on a UV-transilluminator and an imaging gel.

RE-RAPD reactions:

To detect mutations that are found in genotypes, the RE-RAPD reactions were performed on parents only by using 8 prefixes, which were used in the RAPD reactions and the prefixes did not show distinct packages for the species, contained general Main band, considered the main goal in these interactions, the reactions were done in two ways (Salman et al. , 2019) :

DNA slicing:

First: the solutions and materials used and the supplied company:

1. Digestion buffer x 10 buffer solution from promega, U.S.A.
2. Nuclease-free, distilled water supplied by Promega, U.S.A.
3. Clearing enzymes: EcoR1, Hind111, Pest1 supplied by Promega, U.S.A.
4- Actylated BSA Enzyme Serum from Promega, U.S.A.

The method of work:

1. **DNA:** was cut by preparing the reaction in (2) ml tube, the solutions and each enzyme are placed separately, and then the samples are incubated in a water bath of (37) m for a period of (4) hours, to confirm the completion of the segmentation, samples are migrated through the appearance of DNA smeared along the gel.
2. **Double reactions are performed for DNA samples after they are digested:** the same steps as parents were followed for RAPD reactions.
3. **Transfer (separation by gel):** the migration of the products of this indicator, the method used for the migration of the RAPD products was the same except for increasing the concentration of the agarose gel to 2%.

Slicing RAPD products:

This step included (model multiplication + slicing of outputs + posting and detection), needs the same materials and solutions as the first cutting method.

The method of work:

1. The samples were multiplied using PCR in the same way as for RAPD reactions on parents.
2. The PCR products were cut using the three enzymes (EcoR1, Hind111, and Pest1) and each enzyme separately, 8 microliters of the product were taken and placed in a new tube of 2 ml volume containing 5 microliter of distilled water, 2 microliters of enzyme buffer solution for each enzyme are placed, the samples were incubated in a water bath for (4) hours, and then the samples were transferred to freezing until transferring.
3. Samples were carried over on agaros gel at a concentration of 2% in the same way as the first method for RE-RAPD, as well as detection, imaging and saving the images in the computer.

Statistical analysis:

The experience of parents and hybrids, a statistical analysis of all studied traits was performed, according to Random Complete Block Design (R.C.B.D.) With three replications at 5% probability level, to know the differences between genotypes (Al-Rawi and Khalaf Allah, 1980). The

efficiency and discriminant power of RAPD prefixes were estimated, the efficiency of each initiator was estimated using the formula (Grudman et al, 1995):

Efficiency = (No. of band per primer / total number of multiplier bands per primers) × 100

The discriminatory ability was found based on the following formula:

Discriminant power = (number of varying bands per primer / total number of varying band per primers) × 100

The symbols used in Table (4) mean: X1, the molecular genetic distance, X2, the phenotypic genetic distance, X3, the effect of the special federal ability, X4, the strength of the hybrid, for the average of the parents, X5, the power of the hybrid, for the best parents, X6, the average character.

Results and discussion

Evaluation of the performance of genotypes and their individual hybrids for phenotypic traits:

1. Maturity date (day):

Table (4) shows that the highest period for reaching the date of ripening of the plant was for parent 4, reached (145.13) cm, while the minimum period for maturity for the parent 6 it was (129.31) cm, as for hybrids (Table 5), the hybrid 1×5 gave the highest maturity period of (161.56) cm, while the hybrid 4×7 was the lowest maturity period (141.26 cm). Indicates that the aforementioned parents have transferred this trait that surpassed it to their crosses. The results of the general average, the parents had the least period of maturity and reached 138.85 cm, compared with the average of the hybrids that gave the highest maturity period, which reached 150.45 cm., the genotypes (parents and hybrids) that gave the highest time to reach physiological maturity, may give a better chance to make a good vegetation cover and store more material in the stem and leaves, for the length of the photosynthesis, the opportunity to transfer a large amount of foodstuffs to seeds increases during the filling stage, reflected positively on the final product, it is possible to benefit from the parent 4 and the hybrid 5×1 which took the longest time to fill the seeds, to transfer their genes to local cultivars through cross-breeding programs (Al-Jubouri, 2016).

2. Number of branches. plant:

Table 4 indicate that the highest number of branches of a plant was for the parent 4, reached 7.60 branches. plant, while the lowest number of branches for a plant for the parent 5 was 5.40 branches. plant. As for hybrids, the hybrids 1×5 gave the highest number of branches, reaching 10.13 branches. plant (Table 5), while the hybrid reached 3×7, with the lowest branches of the plant reaching 6.93 branches. plant^{-1}, indicates that the aforementioned fathers have transferred this trait that surpassed it to their crosses, the results of the general average, the parents were the least number of branches of the plant and reached 6.92 branches. plant^{-1}, compared to the average of the hybrids that gave the highest number of branches for a plant, which reached 7.80 branches. plant^{-1}, the branching trait in a plant was a genetic traits of the genotype, it was influenced to a large degree by growth factors and this trait is desirable, compensates for the loss of cultivated plants

for any reason, because the increase in branching has a role in giving more pods, reflected positively on the final result (Al-Jubouri, 2014), agreed with Al-Hamdani and Al-Nuaimi (2013).

2. The Leaf area:

Table (4) show that the parent 4 excelled significantly compared to the other parents, recorded the highest average paper area (1632.11) cm^2. $plant^{-1}$, while the parent 5 gave the minimum leaf area of 1233.58 cm^2. $Plant^{-1}$, in individual hybrids (Table 5), the hybrid 1×5 showed a significant superiority and reached 1850.60 cm^2. $Plant^{-1}$ compared to the hybrid 6×4 which gave the least leaf area and reached 1254.81 cm^2. $Plant^{-1}$, indicates that the aforementioned fathers transferred this trait that distinguished them to their crosses, the average of hybrids outperformed and gave the highest average of 1589.12 cm^2. $Plant^{-1}$, compared with the parents' average, it was 1501.87 cm^2. $Plant^{-1}$, the hybrids respond to an increase in leaf area and superiority over their parents, this is an important indicator, as this phenotype is one of the distinguishing features, it has a role in increasing photosynthesis, increase the accumulation of dry matter, reflected positively on the final quotation. As the dry matter is manufactured, which can identify the efficiency of the paper, during the increase in the growth rate per unit weight of the plant from the leaf area, this trait can be used to improve the final yield (Yan and Wallace, 1998).

3. Number of pods. $Plant^{-1}$:

The results of the number of pods trait shown in Tables (4) and (5), the parent 4 was significant excelled, reached 30.13 pods. $Plant^{-1}$, while the parent 5 gave the lowest value for the same trait, which amounted to 24.16 pods. $Plant^{-1}$. As for the hybrids, it gave the hybrid 1×5 a significant advantage, at a rate of 35.10 pods. $Plant^{-1}$, while the hybrid 1×7 gave the lowest averages, at 26.03 pods. $Plant^{-1}$. When comparing the average of parents with the average of hybrids, hybrids were distinguished by a higher value of 30.86 pods. $Plant^{-1}$, while the average for parents was 27.55 pods. $Plant^{-1}$, indicates the response of some parents and their crosses to the increase in the number of pods, as a result of the response of some of parents and their hybrids to the same ratio of holding pods, which has a positive effect on increasing the number of pods, positively affects the final yield in order to obtain high productivity, it must be secured to use genotypes that have a genetic potential that gives a high rate of this trait, considered an important yield component that qualifies it for high production (Al-Ghamdi, 2009).

4. The number of seeds per pod:

Table (4) shows that the parent 4 had a significant superiority in the number of seeds. pod^{-1} and reached 5.28 seeds. pod^{-1}, differed from the parent 5 gave the lowest value amounted to 2.16 seeds. pod^{-1}. As for hybrids (Table 5), the hybrid 1×5 gave a significant advantage, with a rate of 5.20 seeds. pod^{-1}, while the hybrid 1×4 gave the lowest averages, was 3.40 seeds. pod^{-1}, comparing the average of parents with the average of hybrid, hybrids were distinguished by a higher value of 4.28 seeds. pod^{-1}, the average parents gave 3.90 seeds. pod^{-1}, to obtain high productivity, it is necessary to use genotypes that have a genetic potential that gives high production, this trait was an important component of the quotient, contain a large number of seeds. Also, genotypes in which sufficient quantities of dry matter are available during the stage of pod formation and development, may increase the ratio between lush flowers, and cut sterile and aborted flowers, this results in an

increased number of seeds. pod^{-1} per plant, reflected positively on the final product, that the superiority of these parents and their individual hybrids came as a result of their superiority in the quality of maturity, and leaf area, which was positively reflected in the increase in the number of seeds. plant^{-1} trait. The superior genotypes of parents and hybrids can be utilized in crossbreeding programs to improve grain yield (Altin, 2010).

5. Average seed weight (g):

Tables (4) and (5) show the parent 4 significant superior (0.891 g), while parent 5 gave the lowest value (0.691 g). As for the hybrids, it gave the hybrid 1×5 a significant advantage (0.830 g), while the hybrid 1×6 gave the lowest averages (0.830 g). When comparing the average of parents with the hybrids, the parents were distinguished by a higher value of (0.780 g), while for hybrids (0.705 g). The characteristic of the average seed weight (g) is an indication of the efficiency of transport and representation of manufactured materials from the source to the sink at the seed storage sites, it is also an important component of the yield, due to the seed fullness, the superiority of these parents and their hybrids also came as a result of their superiority in the leaf area, led to the transfer and representation of manufactured materials from source to downstream in seed storage sites, reflected positively on the final result (Mille et al., 2005), agreed with Al-Jubouri (2016).

6. Weight of seeds. plant (g):

Tables (4) and (5) show that the parent 4 had a significant superiority (72.74 g) compared to the other parents, while the parent 1 gave the lowest value (32.24 g). As for the hybrids, the hybrid 1×5 gave a significant advantage (72.36 g), while the hybrid 3×6 gave the lowest averages (46.13 g). When comparing the average of parents with the hybrids, the hybrids were distinguished and gave the highest rate (59.61 g), while the average parents gave 52.95 g. The seed. plant weight trait was a major yield component, it was a guide to the conversion efficiency of processed dry materials, the significant difference indicates the response of these crosses to an increase in the average, the superiority of these parents and their individual hybrids was the result of the accumulation of net photosynthesis rate and dry matter in the yield components, reflected positively on the number and weight of the seeds, which positively affected in the final yield.

7. Seed yield (kg. ha^{-1}):

Tables (4) and (5) show that the parent 4 produced the highest average (4122.6 kg. ha^{-1}), while the parent 5 produced the lowest average (2213.6 kg. ha^{-1}). The hybrid 1×5 showed the highest rate (4169.43 kg. ha^{-1}), while the hybrid 4×5 gave a lower yield of (2089.26 kg. ha^{-1}). The average of hybrids was significantly superior to that of the parents, with 3455.65 kg. ha^{-1}, the average for the parents was 2943. kg. ha^{-1}. The grain yield trait was the final result of most of the plant's phenotypic and physiological characteristics, as the increase of this trait and its components is an important achievement for plant breeders, by evaluating the arithmetic means of parents and hybrids, this superiority that came in the yield of seeds came as a result of their superiority in the yield components, reflected positively on the final result. The differences between parents and hybrids are due to the difference in the accumulation of dry matter seeds, and photosynthesis increases during maturity, reflected positively on the final result (Al-Jubouri, 2016).

From the above, it is noticed the superiority of genotype 4 in most of the studied traits, including the seed yield, which can be evaluated in more than a year, and a site to benefit from and adopt it in agriculture, being one of the inputs. It also notes the superiority of the hybrid 1×5 in the quality of the seed yield and most of its components are the characteristic of the number of pods, the number of seeds per pod and the weight of the seeds, the isolated generations of these hybrids can be followed in later generations, and select plants that were superior to these traits.

Genetic relationships between indicators:

1.The genetic relationship between phenotypic and RAPD indicators of parents:

The results indicate the level of phenotypic indicators (based on cluster analysis and average phenotypic characteristics) and RAPD indicators of parents. Parents have a high correlation in terms of genetic distances, the results of the genetic distance of the quantitative characteristics of the parents represented by the pooled scheme were 100% identical to the results of the genetic dimension of the RAPD indicators of the parents. Although the phenotypic indicators were based on the arithmetic mean of the studied quantitative traits, it has been divided into three groups, whereas the RAPD indicators were based on the appearance or absence of the bands of the parents on the agarose gel, likewise, the parent 4 distinguished himself in the highest desirable traits, (Table 4), this parent had the largest number of distinct bands in the RAPD indicators (Table 3), supports the use of molecular markers to classify, diagnose and select traits, because all the traits that appear on the plant. They are caused by the expression of a gene carried on the chromosomes, regardless of other influences. Although the RAPD indicator is random for identifying sites, however, it is of high quality in evaluating the genetic distance of varieties and genotypes, for being able to scan the entire genome, contrary to other indicators, which can only scan 10% of the genome.

2. The genetic relationship between phenotypic and RAPD indices of individual hybrids:

It was indicated by the phenotypic results of individual hybrids, which distinguishes some hybrids through their phenotypic traits, there was a correlation with RAPD indicators on hybrids through the distinctive bands of some hybrids. The hybrid 1×5 was distinguished in its phenotypic traits (Table 5), Have the highest value for 3 traits were number of pods, weight of seeds and seed yield. This hybrid was distinguished by having two bands distinct from all hybrids in the RAPD indicators. The hybrid 2×6 was distinguished for having the highest value of the quantity traits being the number of seeds and seed weight (Table 5), while this hybrid was distinguished for having a distinctive band in the RAPD indicators (Table 3). Likewise, the hybrid 2×4 was distinguished by having the highest arithmetic mean in the characteristic of paper area (Table 5), and it was distinguished by having a distinguished band in the RAPD indicators (Table 3), this indicates that there was a correlation between phenotypic and RAPD indicators, during the results, the distinctive bands of hybrids can be considered a distinguishing of the superiority in the yield traits, because the quantitative traits were governed by a number of genes and during hybridization, these sites were switched on the hybrid, increases the possibility of obtaining variance using RAPD indicators, especially when the correlation between it and the phenotypic indicators was related (Saifuldeen and Raeed , 2021) .

3. The genetic relationship between quantitative traits:

Prepare the evaluation of genotypes and their hybrids, and selection for genotype or hybrid, which have desirable quantitative characteristics at the same time, or the one that can be predicted to have after following subsequent isolated generations. It was revealed through the quantitative traits, as the parent 4 was distinguished, having the highest means of most quantitative traits (Table 4). As for hybrids, the quantitative and qualitative traits varied to the desired direction. The fact that crossbreeding increases the proportion of mixing and obtaining hybrids desirable in terms of quantity and quality. The hybrid 1×5 was distinguished by the highest averages of the studied quantitative traits (Table 5). This supports the correlation between the quantitative characteristics of these hybrids, helps in the success of this program, it is possible to follow the isolated generations of these hybrids only to reach the desired goal in the end. The process of crossbreeding is the primary method for genetic mixing, and obtain desirable varieties with the help of molecular markers to reduce effort, cost and time (Saifuldeen and Raeed , 2021).

4. The genetic relationship between the phenotypic and RE-RAPD indices of the parents:

The use of RE-RAPD markers on Peanut genotypes, considered a successful step with some modifications, during the results obtained from this indicator, the Peanut plant has a high capacity for genetic diversity because 72 genetic mutations were distinguished using only 8 prefixes, this increases the association between phenotypic indicators, because some mutations have a relationship with the quantitative trait. Parent 5 has the highest number of mutant unique packets (Table 3), while same parent has the lowest averages of most of the studied quantitative traits (Table 4), this indicates that most of the mutations that occur were in an undesirable direction, the reason was that the mutations cause a defect in one of the genes responsible for those quantitative traits, which affects the quantity of the trait (Saifuldeen and Raeed , 2020) .

5. The genetic relationship between RAPD indicators and RE-RAPD indicators for parents:

The RAPD indicator is considered in this paper, the RFLP indicators were based, became supportive of the results of the first indicator, the results of the RAPD tend to be comprehensive. An index can be used to check the discrepancies within the pieces that multiplied in the first index, a turning point was found in this research, it was not previously supported, can be used with a small number of prefixes may be 5 primers only, results were general for most packages. An index can be used to detect its contrast, this increases the correlation between those molecular indicators of different specialties. These results were in agreement with many researchers, including Zsubori et al., (2003); Xuxiao and others, (2009), Wilson et al., (1991), Torres et al., (2006) and Al-Skmani, (2017).

Correlation coefficient:

Table (6) show that the adjective maturity date, the correlation coefficient was positive and highly significant, indicate the effect values of the unite special estimator, the strength of a hybrid for average parents was better parents, the average trait and between the hybrid strength of average parents. A hybrid strength for best parents and average trait, and between the hybrid strength of the best parents and the average trait, respectively, reached 0.781, 0.770, 0.820, 0.934, 0.913 and

0.881 respectively. As for the probability level of 5%, it was positive only between the phenotypic genetic dimension and the effect of the special federal estimate and reached 0.457. Note that all the correlations that were not mentioned were positive or negative, but did not reach the limits of statistical significance.

While in the adjective number of branches per plant, the correlation coefficient was between the effect values of the unite special estimator, hybrid strength of average parent, the average of the trait and between the hybrid strength of the average parent and the average of the trait, was positive and spirited, reached 0.798, 0.731, 0.759, 0.743 and 0.782, respectively. At the 5% probability level, it was positive only between the phenotypic genetic distance, the effect of the special unite estimate was 0.434. Note that all the connections that were not mentioned were positive or negative, but not reach the limits of the statistical significance.

The leaf area trait was the correlation coefficient, indicate the effect values of the unite special estimator, the hybrid strength of average parent and average trait, between the hybrid strength of average parents and average trait, was positive and spirited (0.795, 0.921, 0.931, and 0.423), respectively. At the 5% probability level, it was only negative between the phenotypic genetic distance, the effect of the special unite capacity amounted to (-0.456). Note that all the connections that were not mentioned were positive or negative, but not reach the limits of the statistical significance (Raeed and Saifuldeen , 2020) .

The number of pods. Plant, the correlation coefficient was between the effect values of the unite special estimator, and hybrid strength of average parent, hybrid strength for best parents, showed the strength of the cross between the average parents, the strength of the cross between the best parents, and the average trait, it showed the strength of the hybrid about the best parents, the average of the trait is positive and high in significance and reached 0.723, 0.818, 0.940, 0.627 and 0.755, respectively. As for the probability level of 5%, it was positive only between the special ability and the average trait and reached 0.501, and negative and significant only between the molecular genetic distance, the effect of the special federal capacity amounted to -0.419. Note that all the connections that were not mentioned were positive or negative, but not reach the limits of the statistical significance.

Number of seeds. pod, the correlation coefficient was positive and highly significant, indicate the effect values of the unite special estimator, hybrid strength for average parents, the strength of a hybrid was the best parents and the average trait, and between the strength of the cross between the average parents and the power of the cross between the best parents, the average of the trait, the strength of the hybrid for the best parents, and the average of the trait, it was positive and significant and reached 0.710, 0.701, 0.899, 0.923, 0.711 and 0.752, respectively. At the 5% probability level, it was positive only between the phenotypic genetic distance, the impact of the special unite capacity reached 0.457. Note that all the connections that were not mentioned were positive or negative, but not reach the limits of the statistical significance.

The seed weight trait was the correlation coefficient, indicate the effect values of the unite special estimator, and the strength of the hybrid for the average parents, and the power of the hybrid for the best parents, and the average trait and between the hybrid strength of average parents, the best

parent and average trait, and between the strength of the hybrid about the best parents and the average trait, it was positive and significant and reached 0.798, 0.854, 0.793, 0.793, 0.935 and 0.887, respectively. At the 5% probability level, it was only negative between the phenotypic genetic distance, and the effect of the special unite ability amounted to -0.476. Note that all the connections that were not mentioned were positive or negative, but not reach the limits of the statistical significance.

The correlation coefficient for a trait shows the weight of seeds. plant (g), the correlation was between the effect values of the special unite capability, the average trait and the hybrid strength, for average parents and a strong crossbreed for better parents, and hybrid strength for the best parents, the average trait was positive and high in significance, reached 0.899, 0.819 and 0.874, respectively. Note that all the correlations that were not mentioned were positive or negative, but not reach the limits of the statistical significance.

The seed yield (kg. ha^{-1}), the correlation coefficient was positive and highly significant, show the effect values of the special unite capacity, and the hybrid strength of average parent and average trait, between the hybrid strength of average parents, the strength of the hybrid for the best parents is positive and high in significant, reached 0.631, 0.943 and 0.960, respectively. Note that all the correlations, that were not mentioned and for all the studied traits were positive or negative, but not reach the limits of the statistical significance.

These results were agreement with many researchers, including Xuxiao et al., (2009); Wilson et al., (1991); Torres et al., (2006); Al-Sakmani (2017) and Al-Zuhairi (2014).

Table (1) The primers used in the RAPD-PCR study for parents + hybrids and the RE-RAPD, as the + + sign indicates the use of the primer.

Primers	The primers relay - 3 - 5	For parents only RAPD-PCR	On parents + hybrids RAPD-PCR	RE-RAPD reaction
SRA- 13	AGCTCCGTCA	+	+	
SRF -16	CTGTGCTCCA	+		
SRA-11	CGCATCGTCA	+		
SRO -06	TCTGCGATCC	+	+	
SRD -02	CACAGCGACC	+		+
SRO 12	GTGCACCCAC	+		
SRM -10	GTATAACTGG	+	+	
SRE -20	CGATCGTCGT	+		
SRA -20	GAACGGGAAG	+		
SRD -03	TGACTCAACC	+		+
SRG -13	CAGTCATGTG	+	+	
SRD -08	CATGGCGCAC	+		
SRW -13	CACAGCGACA	+	+	
SRP -01	GAAGCACTCC	+	+	
SRG -11	CGCTCAGCTC	+	+	+

SRQ -15	CAGTGCATCT	+		
SRB -20	CGACTCAACC	+	+	+
SRB -10	TTCTCATGGT	+	+	
SRH-01	CACTAGGATG	+	+	
SRD -02	CGCAAGTCGT	+		
SRJ -14	CGATGACGTG	+	+	+
SRJ -12	CCAGCATTAC	+		
SRO -04	CAGCTGGGAC	+	+	+
SRW -08	TAAAAGAGAA	+		
SRAB-12	GCTAAATCGA	+		
SRH-08	TGGACACCCC	+		+
SRN-10	ACTACTCAAG	+		
SRO-05	ATCAGTCACT	+		

Table (2) Components of segmentation of genomic DNA by cutting enzymes ECOR1, Hind111, Pest1.

No.	Solution	The final concentration	Maekerolatr / model
1	Sterile distilled water		16.80
2	Digestion buffer buffer × 10	X1	2.00
3	Actylated BSA		0.20
4	DNA. Genomic DNA	1 mcg	20.00
5	Enzyme shredder	10 units	1.00
6	Final volume		40.00

Table (3) number of sites, the molecular sizes, number of bands and distinctive bands, contrast ratio, efficiency, discriminant ability and polymorphism of the primers used in the study of hybrids.

No.	Primer name	Molecular size	No. sites produced	No. disparate sites	No. general sites	No. primers bands	No. disparate bands	No. general bands	No. unique bands	No. absent bands	Contrast ratio %	Primer efficiency	Discriminatory	Formal pluralism
1	SRf -24	175-1300bp	5.9	7.3	7.2	87	-	-	36	136	173	1	8	9
2	SRr-02	200-2500bp	6.1	7.6	7.7	90	-	1	36	140	173	-	10	12

3	SRw - 01	175-2250bp	9.8	12.1	9.8	100	3	-	-	223	222	-	12	12
4	SRc - 12	475-1500bp	4.5	5.5	4.5	100	-	-	-	103	106	1	8	9
5	SRF-16	600-2500bp	3	3.7	3	71	1	-	-	69	69	-	3	7
6	SRW - 08	175-950bp	4.6	5.7	8.2	60	-	-	72	105	147	2	7	10
7	SRt-14	425-1600bp	7.8	9.6	7.8	88	-	-	-	178	168	-	12	12
8	SRh - 14	425-1800bp	5.8	7.2	7.4	90	-	1	36	134	160	1	9	11
9	SRv - 15	450-1000bp	6.5	7.7	6.5	100	-	-	-	143	148	-	7	9
10	SRd-13	150-1200bp	4.8	5.9	8	100	-		72	110	186	2	6	8
11	SRs -13	500-1300bp	0.9	1.1	4.1	77	-	1	72	22	95	2	4	6
12	SRy - 13	350-1500bp	3.7	4.6	5.3	100	1	1	36	86	132	1	8	10
13	SRe-11	150-900bp	4.4	5.4	4.4	100	1	-	-	101	111	-	7	7
14	SRd-15	300-1100bp	2.1	2.6	3.6	83	-	1	36	48	94	1	5	7
15	SRm-55	200-800bp	2.7	3.4	2.7	100	1	-	-	63	66	-	6	5
16	SRg - 42	350-1000bp	3.1	4.1	3.3	75	-	1	36	40	74	1	3	5
17	SRW - 13	200-1000bp	4.1	5	5.6	88	1	3	36	93	129	1	9	9
Total		150-2500bp	80.9		0	88	8	10	432	1840	2253	13	124	148

Ann. For. Res. 65(1): 6342-6359, 2022
ISSN: 18448135, 20652445

ANNALS OF FOREST RESEARCH
DOI: 10.5281/zenodo.7410202

Table (4) Parents' averages for the studied traits.

Traits / Parents	Maturity date (day)	No. Branches. plant	Leaf area	No. pods	No. seeds	mean Seed weight	Seeds weight	Seed yield
1	143.52	7.03	1538.42	26.9	4.93	0.761	32.24	1786.7
2	131.43	5.53	1578.55	29.6	2.46	0.811	71.75	3611.5
3	143.32	7.30	1471.14	27.1	4.38	0.780	61.13	3320.8
4	145.13	7.60	1632.11	30.13	5.28	0.891	72.74	4122.6
5	141.43	5.40	1233.58	24.16	2.16	0.691	37.94	2213.6
6	129.31	7.36	1534.51	26.16	3.89	0.798	41.16	2234.5
7	137.83	7.16	1524.81	28.85	4.66	0.845	63.7	3216.2
Mean	**138.85**	**6.92**	**1501.87**	**27.55**	**3.90**	**0.780**	**52.95**	**2943.7**

Table (5) Hybrids' averages for the studied traits.

Traits / Hybrids	Maturity date (day)	No. Branches. plant	Leaf area	No. pods	No. seeds	mean Seed weight	Seeds weight	Seed yield
1×2	145.30	8.36	1540.76	31.83	4.53	0.711	50.56	3290.35
1×3	143.93	7.63	1558.83	27.26	4.53	0.629	57.40	3048.22
1×4	154.60	7.66	1574.53	26.06	3.40	0.783	53.05	3137.16
1×5	161.56	10.13	1850.60	35.10	5.20	0.830	72.36	4169.43
1×6	154.20	7.10	1475.76	28.73	4.66	0.595	53.32	2892.81
1×7	157.76	7.50	1746.23	26.03	4.50	0.749	49.96	3904.10
2×3	153.23	7.30	1681.33	30.13	4.16	0.752	63.08	3029.69
2×4	161.51	8.36	1593.46	28.40	4.30	0.596	59.42	2089.28
2×5	153.20	7.10	1376.16	28.90	4.03	0.650	65.17	3283.43
2×6	145.60	7.30	1522.70	31.03	3.76	0.711	65.47	3816.03
2×7	147.60	9.16	1630.96	31.86	4.26	0.770	48.13	3415.95
3×4	144.46	7.33	1577.43	29.76	4.80	0.789	70.88	3585.02
3×5	152.06	7.46	1737.46	31.73	3.80	0.716	65.47	3918.96
3×6	146.66	7.13	1735.90	32.43	5.26	0.639	46.13	3904.10
3×7	143.36	6.93	1759.70	33.06	3.96	0.619	61.52	3029.69
4×5	145.06	7.50	1723.46	31.36	4.20	0.601	55.51	2089.26
4×6	155.30	7.30	1254.81	37.53	3.93	0.804	71.03	4079.18
4×7	141.26	8.36	1478.70	39.06	4.23	0.743	64.40	3824.84
5×6	143.93	8.60	1723.46	31.36	4.16	0.711	61.90	4040.27
5×7	154.60	7.66	1254.86	27.53	4.30	0.629	55.73	4096.94
6×7	154.30	8.13	1574.53	29.06	4.03	0.783	61.52	3923.95
Means	**150.45**	**7.80**	**1589.12**	**30.86**	**4.28**	**0.705**	**59.61**	**3455.65**

Ann. For. Res. 65(1): 6342-6359, 2022
ISSN: 18448135, 20652445

ANNALS OF FOREST RESEARCH
DOI: 10.5281/zenodo.7410202

Table (6) The correlation factor between the molecular genetic distance, the phenotypic distance, the effect of the unite special ability, the strength of the hybrid vigor for the average parents, the strength of the hybrid for the best parents, and the average for the traits.

Correlations	Maturity date (day)	No. Branches. plant	Leaf area	No. pods	No. seeds	mean Seed weight	Seeds weight	Seed yield
r_{x1x2}	-0.172	0.072	-0.172	-0.172	-0.172	-0.172	-0.172	-0.172
r_{x1x3}	-0.322	0.162	-0.025	-0.419*	0.162	0.238	-0.016	-0.108
r_{x1x4}	-0.307	0.147	0.071	-0.186	0.147	0.110	0.100	0.118
r_{x1x5}	-0.271	0.266	-0.132	-0.112	0.266	0.138	0.305	0.311
r_{x1x6}	-0.240	0.128	0.072	-0.073	0.128	0.126	-0.054	-0.027
r_{x2x3}	0.438*	0.434*	0.723*	0.141	-0.457*	-0.476	-0.107	0.143
r_{x2x4}	0.024	-0.164	-0.299	0.039	-0.164	0.098	-0.021	0.239
r_{x2x5}	-0.061	-0.299	-0.005	-0.035	-0.299	0.063	-0.275	-0.031
r_{x2x6}	-0.057	-0.279	-0.325	-0.110	-0.279	0.068	-0.105	0.155
r_{x3x4}	0.781**	0.798**	0.835**	0.723**	0.710**	0.798**	0.302	0.631**
r_{x3x5}	0.770**	0.731**	0.230	0.818**	0.701**	0.854**	0.178	0.218*
r_{x3x6}	0.820**	0.759**	0.921**	0.501*	0.899**	0.793**	0.899**	0.943**
r_{x4x5}	0.934**	0.743**	0.249	0.940**	0.923**	0.975**	0.819**	0.960**
r_{x4x6}	0.913**	0.791**	0.931**	0.627**	0.711**	0.935**	0.234	0.208*
r_{x5x6}	0.881**	0.782**	0.180	0.755**	0.752**	0.887**	0.874**	0.361

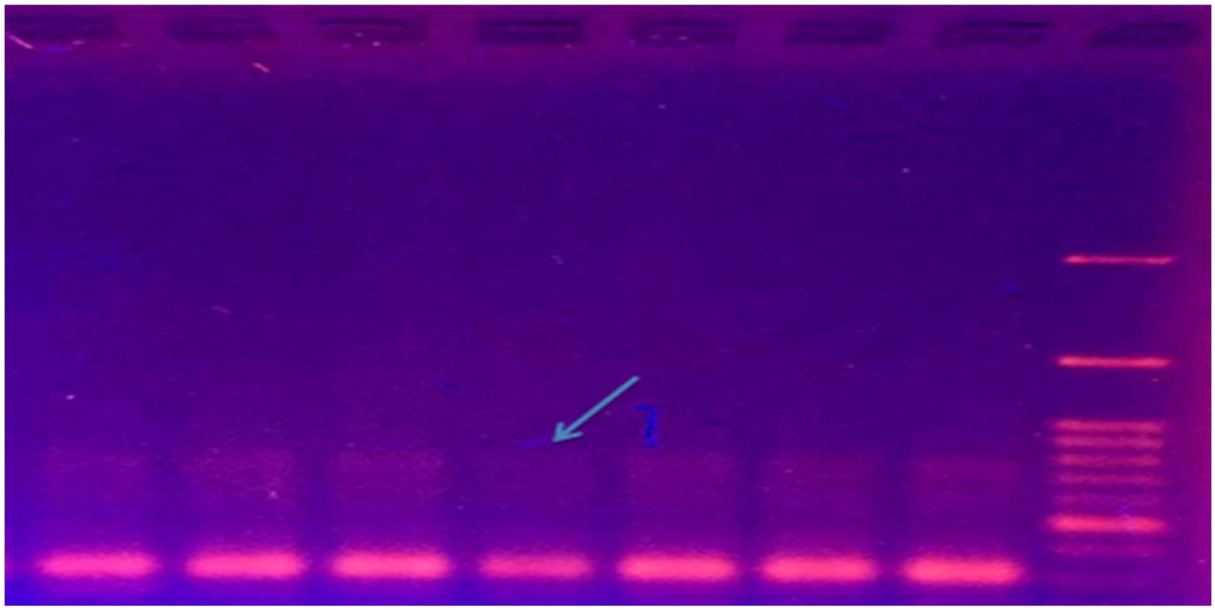

Image (1) Primer multiplication products with the DNA of the seven parents and the stage on an acarose gel at a concentration of 1.5% with the volumetric index M.

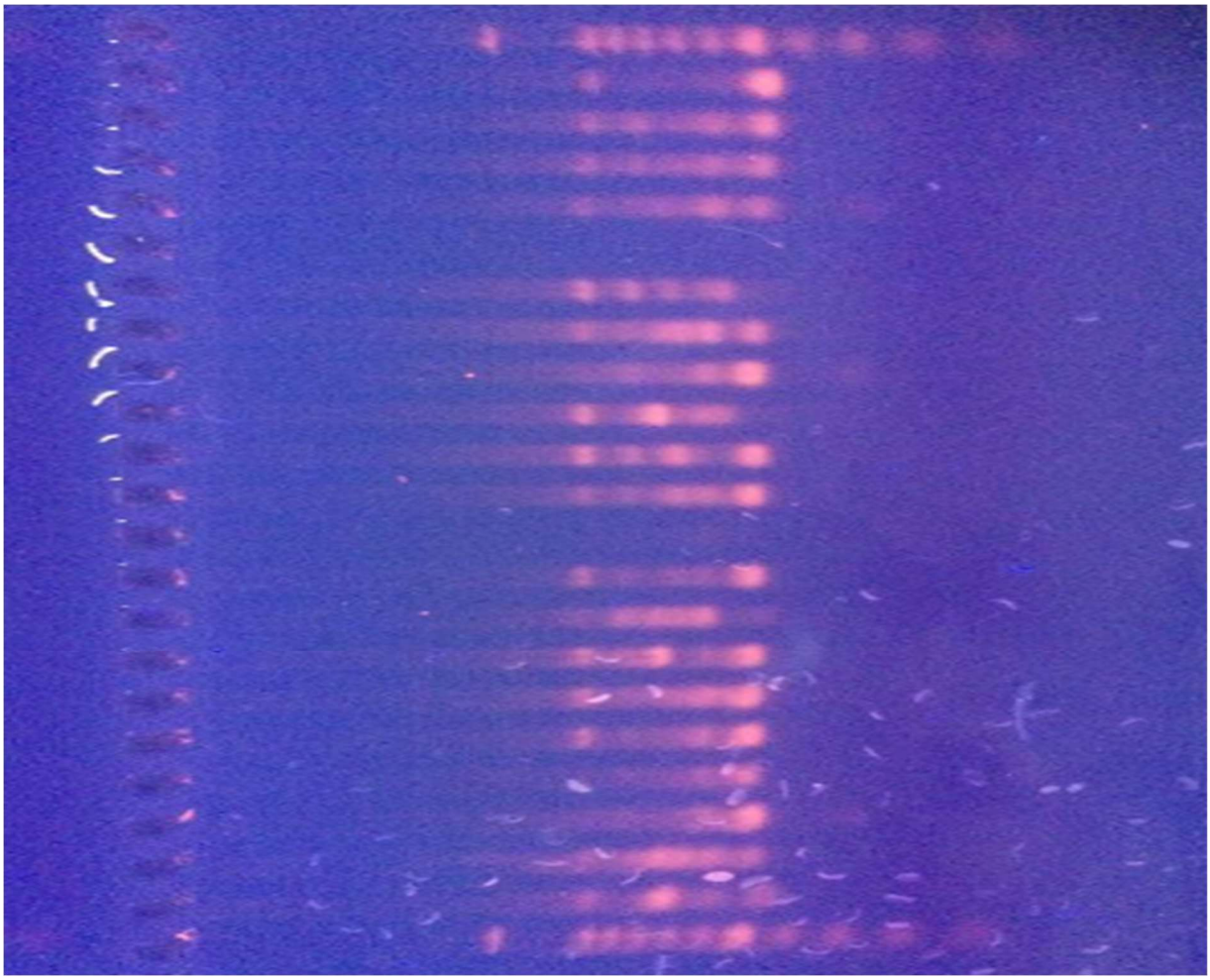

Image (2) Primer multiplication products with 21 single hybrid DNA of the first generation and phase on an acarose gel at a concentration of 1.5% with a volumetric index M **.**

References

Al-Ghamdi S.S.H. (2009). The application of biotechnology to improve field crops (beans). PhD thesis. College of Food and Agricultural Sciences, King Saud University, Saudi Arabia.

Al-Hamdani S.Y.H. and M.H.M. Al-Nuaimi (2013). Genetic degradation and some genetic parameters of the growth and yield of second-generation hybrids in Peanut. *Kufa Journal of Agricultural Sciences*. 5 (1): 347-383.

Al-Jubouri, R.M.A. (2016). Genetic analysis and evaluation of some promising genotypes and the synthetic variety derived from them to tolerate salinity in pea (Vicia faba L.). PhD thesis, Faculty of Agriculture, University of Tikrit.

Al-Rawi K.M. and A. Khalaf Allah (1980). Design and analysis of agricultural experiments. Ministry of Higher Education and Scientific Research. University of Al Mosul .

Al-Skmani, R.Z.M. (2017). The use of phenotypic and molecular indicators to assess the genetic performance of a number of genotypes of pea plants and their individual hybrids (*Vicia faba* L.), PhD thesis, College of Education for Pure Sciences, University of Tikrit.

Altin S.K. (2010). Heritabilities, gains from selection and genetic correlations for grain yield of barley grown in two contrasting environments Barley Genetic. *Newsletter*. 22:6-13.

Al-Zuhairi, N.S.A. (2014). The nature of genotypes using single, triple and even hybrids between pure strains of yellow maize (Zea mays L.) and predicting the characteristics of paired hybrids. PhD thesis, Faculty of Agriculture and Forestry, University of Mosul.

Grudman H., Schneider C., Hartung D., Daschner F.D. and Pith T.L. (1995). Discriminatory power of three DNA Typing techniques for Plant aeruginosn. *J. clin. Microbiol,* 3: 528-532.

Hedrick P.W. (2005). Genetics of populations, Jones and Bartlett, London, UK. *J. Trop. Agric. Sci.*, 26(1): 27-33.

Li P., Wang Y., Sun X. and Han J. (2009). Using microsatellite (SSR) and morphological markers to assess the genetic diversity of 12 falcata (*Medicago sativa* spp. *falcata*) populations from Eurasia. *African Journal of Biotechnology*, 8: 2102-2108.

Mille B., Belhajfraj M., Monod H. and de vallavieille pope C. (2005). Assessing. Four-way mixtures of winter wheat cultivars from the performances of their two- way and Individual components . *European Journal of plant pathology*. 44 (2): 163-173.

Raeed Mejbel Abdullah and Saifuldeen Ahmed Hasan . 2020 . Estimation of components of genetic variance using Jinks-Hayman method analysis on the crop of faba bean (*Vicia faba* L.) . Int. J. Agricult. Stat. Sci. 16(1); pp. 1897-1903 .

Sabouh, M., Hadid M.L. and Qanbar A. (2010). Quantitative Genetics (theoretical part), Faculty of Agricultural Engineering, University of Damascus.

Saifuldeen Ahmed Hasan and Raeed Mejbel Abdullah . 2020 . Estimating the performance and gene action of a number of individual genotypes and hybrids on the crop of faba bean (*Vicia faba* L.) . Plant Archives , 20(2) ; pp. 8981-8988 .

Saifuldeen Ahmed Hasan and Raeed Mejbel Abdullah . 2021 . Characterization of Genetic Variability Through The use of Rapds Markers, of A Group of Native and Commercial Genotypes of Bean Species . . Int. J. Agricult. Stat. Sci. 17(1) ; pp. 1817-1703 .

Salman Dastan, Behzad Ghareyazie, Seyyed Hasan Pishgar . (2019). Environmental impacts of transgenic Bt rice and non-Bt rice cultivars in northern Iran. Biocatalysis and Agricultural Biotechnology . Volume 20, July 2019, 101160 .

Sardana, S., Mahajan R.K., Gautam N.K. and Ram B. 2007. Genetic variability in pea (*Pisum sativum* L.) germplasm for utilization. *SABRAO J. Breed. and Genet.*, 39 (10) : 31-41.

Torres A.M., Román B., Avila C.M., Satovic Z., Rubiales D., Sillero J.C., Cubero J.I. and Moreno M.T. (2006). Faba bean breeding for resistance against biotic stresses: towards application of marker technology. *Euphytica* 147:67–80.

Wilson G.G. and Murray, N.E. (1991).Restriction and Modification Systems. *Annu. Rev. Genet.* 25: 585–627.

Xuxiao Z., Liw J., Guan S., Wang Q., Liu G., Jerrey P. and Redden R. (2009). Molecular variation among Chinese and global winter faba bean germplasm. *Teor. Appl. Genet*, 118: 971-978.

Yan W. and Wallace D.H. (1998). Plant reeding and whole system crop physiology CAB int1.,198 Mad.Are.N.Y.USA.pp.390.

Zahra Aminfar Babak Rabiei Masoud Tohidfar Mohammad Hossein Mirjalili. (2019). Selection and validation of reference genes for quantitative real-time PCR in Rosmarinus officinalis L. in various tissues and under elicitation . Biocatalysis and Agricultural Biotechnology . Mendeley Data, V1, doi: 10.17632/9g5j49rygw.1 .

Zsubori Z., Gynenes Z., Hegy O.I., Pok I., Racz F. and Szoke C. (2003). Inheritance of plant and ear height in maize (*Zea mays* L.). Agricultural Research Institute of the Hungarian Academy of Sciences. *Martonvasar* . P: 1-4.