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MOLECULAR PREDICTION FOR THE IDENTIFICATION OF TANNINS IN A NUMBER OF GENOTYPES OF (*VICIA FABA* L.) AND THEIR DI ALLELES CROSSING AND COMPARE RESULTS QUANTITATIVELY

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Abstract

The aim of this research is to determine the early concentration of tannins for eight pure herbaceous genotypes obtained from ICARDA and its individual hybrids for the first generation in order to determine the desirable fathers and hybrids that are free of tannins using molecular markers by means of correlations with tannin concentration. RAPD-PCR markers and comparing the molecular results with the concentration of tannins in the seeds and linking them with the desired crop traits during breeding program to obtain a desired variety suitable for production in terms of qualitative and quantitative qualities. The number genotypes and hybrids were transplanted randomly and DNA was extracted from the young leaves of the samples after (45) days of transplantation. The concentration and purity of DNA. RAPD-PCR markers were applied to the DNA of the eight genotypes and their individual hybrids using (6) primers. With the concentration of tannin either gravitational or incompatible with the apparent beams and phase on the gel electrophoresis and samples were dyed with ethidium bromide and revealed on ultraviolet radiation and photographed with a digital camera and images were preserved by computer and analysis of the results, and after the arrival of the crop to the stage of maturity took the seeds and dried The mill was measured concentration Altanien where then compared the results of the concentration Altanien with molecular results . Molecular results and comparison with the concentration of tannins in the seeds showed that the best primers used in determining the low tannins in the parents were the OPJ-14 primer because it corresponds with the estimate of the tannins in the parents at 100%. The efficiency of OPG-11 was high in the identification of parents, where the parents identified P1, P8 and other primers followed in the diagnosis of parents, while in the camel, the OPG-11 primer (4) hybrids of low hybrids of the second (6) and one medium They are (13,16,18,24,48,58) as well as the OPJ-14 primer the (4) hybrids of low-tannins camel and hybrid of medium-tannins High tannin hybrids are (18,24,78,35,37,48) as well as OPJ-12 primer the (3) low-tannin hybrids, high-tannin hybrids and average tannin hybrids (13,18,23,24,67,58) as well as prefixes The other rolled in the diagnosis . The number of genotypes and hybrids were divided into three categories of tannin concentration: the low-tannin group, which contains pure mutant genes, eliminating the role of the wild-type (high-tannin) gene. To reduce the concentration of tannins in half, the high-tannin model contains a pure wild gene, which increased the concentration of tannin. The molecular diagnosis based on RAPD primers gives positive diagnosis of the mutant model on parents and hybrids of both low and middle classes of mutant gene in both.

Keywords : Tannins, RAPD-PCR, *Vicia faba*

Introduction

The leguminous (*Vicia faba*) L returns to the fabaceae and is considered one of its most important crops because its seeds contain a high percentage of protein ranging between 23-37% as well as it contains quantities of sugary and starchy materials and some vitamins Protein in forage diets (Cre´pon, *et al.*, 2010). In addition to its role in improving soil properties by stabilizing atmospheric nitrogen with the root bacteria bacterial that coexist with it ((FAO, 2003). The bean plant contains a group of amino acids, carbohydrates, vitamins and fatty substances Dissolved, the crop is used on top Some conditions have been reported, such as kidney failure, kidney stones, liver impairment and eye diseases (El-Bramawy, *et al.*, 2012).

The tannins are considered a complex phenolic compound with a molecular size that ranges between (3000-20000) Dalton, and it is considered the condensed tannin in legumes. The molecular composition of (5-7) aromatic rings with (12-16) phenol group (Alonso *et al.*, 2000). The second one is estimated in the seeds of the pea, by marinating the powder from the seeds and treating them with Vanillin-HCL according to the method mentioned (Price, *et al.*, 1978). The ratio is calculated based on the color of the solution by the spectrophotometer in the wavelength (500nm). The high concentration of high tannins in beans is more than 20% - 46% and the low concentration of high tannins is between 1% -10% (Abdulrahim *et al.*, 2004; Singh, *et al.*, 2012).

The second is associated with proteins during cooking and digestion, and the second inhibits digestive enzymes during metabolic processes (Marquardt, 1989). Through continuous research in determining the concentration of the tannins in the beans, it was found that the high-proportion tannins have a correlation with appearance (the color of the flower crown) where it is colored when the tannins are high, and white when the tannins are low, and many traditional studies have been conducted in this field until they are reached The researchers sought to identify a recessive gene linked to the low tannins ratio zt1, zt2)) resulting from a mutation in the original gene Wield gene mutation, and this trait is related to and determined by the color of the flower (Gutierrez *et al.*, 2007).

In the study he undertook Grosjean *et al.* (2000) to conduct a strike between the lines of the hereditary high-tannin and the hereditary lines of the low-tannins where the first generation was all high-tannins and in the second generation the ratio was (3: 1) this markers that it is a recessive trait and is inherited according to Mendel inheritance. He emphasized Williams *et al.* (1990) that the first gene ((zt1) can be distinguished from the second gene (zt2) by the proportion of the second which is less in the

second gene and the color of the flower in the first gene is bright white.

It is difficult to study traceability of the second with other cropping traits, relying solely on traditional studies, and it takes a long period of time to conduct crosses (Crofton *et al.*, 2000). Therefore, researchers in this field have developed the use of Molecular markers in identifying the second, so the use of the Marker assisted selection (MAS) was very useful in breeding programs to assess seed quality. But through the RAPD markers, all the obstacles that accompany the phenotypic diagnosis were resolved, and several starting parameters related to the presence of tannins and absences were identified, and it is recognized that the RAPD indicators are easy, low cost, fast results, and less affected by the environment that helped a lot in the success of the education program in predicting the other Even before planting (Avila *et al.*, 2003; Silva *et al.*, 2003; Sugita *et al.*, 2004; Liu *et al.*, 2005). SCAR markers were used using RAPD markers and gave good results in early diagnosis of tannin. Therefore, the aim of this research was to determine the tannins in the bean plant based on molecular markers and to compare these results with the results of tannin concentration in the seeds.

Materials and Methods

1- Sample collection

The genetics of (36) genotypes (eight parents and (28) hybrids) were cultivated in the nursery of the Kirkuk Agricultural Directorate on October 25 2015, and samples were collected from the plants a month and a half after the date of planting from all the genotypes where ((4-5) Young leaves from the growing top were placed in special tagged bags and transferred directly to the laboratory for DNA isolation.

Table 1 : Genotypes used in the study of parents, symbols and hybrid symbols**.**

Origin		Pedigree						Entry			Symbol
ICARDA		2000/DSO/0405-HBP/7005-2/B7/ DT						PO6-OO1FB / FL			P1
ICARDA		2000/DSO/0405-HBP/7841/B7/ DT						PO6-OO2FB / FL			P2
ICARDA		2000/DSO/0405-HBP/7106-1/B7/ DT						PO6-OO3FB / FL			P3
ICARDA		2000/DSO/0405-HBP/7380/B7/ DT						PO6-OO5FB / FL			P4
ICARDA		Selection from ILB 1814						PO6-OO9FB / FL			P5
ICARDA		2000/DSO/0405-HBP/7038/B7/ DT						PO6-O11FB / FL			P6
ICARDA		2000/DSO/0405-HBP/7486/B7/ DT						PO6-O13FB / FL			P7
ICARDA		0405-SP80B(DS)/7986/B7/ DT						PO6-O14FB / FL			P8
Hybrid	Symbol	Hybrid	Symbol	Hybrid	Symbol	Hybrid	Symbol	Hybrid	Symbol	Hybrid	Symbol الرمز
1×2	12	1×7	17	2×6	26	3×6	36	4×7	47	6×7	67
1×3	13	1×8	18	2×7	27	3×7	37	4×8	48	6×8	68
1×4	14	2×3	23	2×8	28	3×8	38	5×6	56	7×8	78
1×5	15	2×4	24	3×4	34	4×5	45	5×7	57		
1×6	16	2×5	25	3×5	35	4×6	46	5×8	58		

2-Genomic DNA extraction

The DNA was extracted from the young leaves according to the method (CTAB) according to what he mentioned (Weigand, *et al.*, 1993; Huang, *et al.*, 2013). The DNA was purified using a new method developed in this research, modified from the isolation method. The DNA concentration and purity was measured using the Nano drop system.

3-Conducting RAPD-PCR markers to diagnose the tannins

Table 2 : Primers used to diagnose tannin concentration

The name of the prime	Primer sequence	Recessive gene	Molecular size For band	Band type
OPC -05	GATGACCGCC	Zt1	551bp	Appearance
OPG- 11	TGCCCGTCGT	Zt1	1171bp	Absent
OPG -15	ACTGGGACTC	Zt2	600bp	Appearance
OPQ -15	GGGTAACGTG	Zt2	476bp	Appearance
OPJ -12	GTCCCGTGGT	Zt2	886bp	Absent
OPJ – 14	CACCCGGATG	Zt2	504 bp	Absent

The isolated genomic DNA was used from the parents and individual hybrids, and the concentration of tannin in the seeds of pea under study was predicted by relying on the RAPD method using (6) primers and each bands initiator was special and that the presence or absence of this bands markers the rise or decrease of tannin as shown in the table (2) According to The road mentioned by (Gutierrez *et al.,* 2007; Gutierrez *et al.*, 2006; Gutierrez *et al.*, 2008).

The RAPD markers of the studied samples were performed according to the method mentioned by Williams *et al.* (1990) with some modifications as shown in Table No. (3). The main reaction mixture was prepared by mixing the reaction components in a 2 ml sterile Eppendroffe tube and the mixture was discarded in a Microfuge device for 3-5 seconds to complete the mixing of the reaction components, taking into account that the work inside the Hood is sterile, wear gloves and put tubes in an ice As shown in the following table (3)

Table 3 : Solutions used in the RAPD markers

C	Components	Volume
1	Green Master mix	12.5 µ l
2	Primer	2 µ l
3	Nuclease free water	8.5µ l
4	DNA template	2µ l
5	Total Volume	25µ l

RAPD-PCR program performed by the following: pre denaturation 1 cycle at 94 ºC for 7 min, 40 cycle (denaturation 93 ºC for 45 sec; annealing 36 ºC for 1 min ; extension 72 ºC for 1.5 min) and final extension 1 cycle at 72 ºC for 7 min. After the PCR amplifications program was finished, 5µL of PCR products were separated using gel electrophoresis in a concentration of 1.5% with DNA marker, after migration the gel stained by ethidium bromide for 60 min and visualized under UV- trans illuminator. And pictures of jellies using a high-resolution digital camera, the pictures were transferred to the computer for statistical analysis later.

4 -Estimating the tannin in the seed

Tannins were estimated in the seeds of the leguminous plant according to the method of Vanillin-HCL, according to the method mentioned (Price *et al.*, 1978). The ratio is calculated based on the color of the solution by the spectrophotometer in the wavelength (nm 500) (Ingh, *et al.*, 2012).

First: Materials used: HCL hydraulic solution, Vanillin powder, Methanol, distilled water**,**

Second: The solutions used: 1 **%**HCl solution in methanol (V / V), vanillin-HCl solution **(** Vanillin in methanol and 8% HCl in methanol1%), Device Calibration Solution Blank (4% HCL-Methanol).

Third: The devices used: Blender, Crusher & Seed Blender, Balance Sensor, Water bath, 4000 Centrifuge, Spectrophotometer, Computer.

Fourth: How it works :(5) Dry seeds were taken after harvesting and drying for each parent and camel model. Then the seeds were ground using a blender until a fine powder was obtained. I took (0.2) g of the powder and put it in a test tube and (10) ml of the 1% HCl in methanol (V / V) solution prepared in advance was added and the samples were shaken for ten minutes to dissolve the powder in the solution. Samples were centrifuged centrally for two minutes at a speed of (2500) rpm and raised quietly. Take one of Supernatant and add to a new tube containing (5, l) of previously banned vanillin-HCl solution with stirring for one minute. The samples were incubated in a water bath for (30) minutes at (37) C. The sample was placed in the spectrometer in the designated place after calibrating the apparatus in a blank calibration solution at a wavelength of 500nm and connecting the device on the computer to record the reading and save it**.**

Fifth: How to calculate the concentration of tannin in seeds using the Catechin equivalent (C.E) equation, which states that**:**

C.E. % ¬¬ = 200/100 x 10x C, C: represents the device's reading of the sample at the wavelength of 500nm, 10: represents the sample dilution, 200: represents the sample size taken in mg.

Results and Discussion

1-Molecular results for the tannins

The results of the second are considered one of the most important qualitative results obtained for the genotypes of the leguminous plant under study. This study is considered one of the pioneering studies in this field in partially tracking the path of the second and its estimation in seeds and finding the relationship between them, because the characteristic of the presence of tannin in the seeds of legumin has health and economic problems. Several literature has been published by researchers in this field aiming to devise pure varieties or strains containing a small or no percentage of tannin in the seeds. The discovery (Gutierrez *et al.* 2007; Gutierrez *et al.*, 2006; Gutierrez *et al.*, 2008) of the presence of two mutant mutation genes in the leguminous plant gives a lower level of the Tannin than the wild style Dominant gene which These two genes are highly zt1 and zt2, and these Mendelian genetics are followed in the method of heredity. These genes are not the main responsible for the production of the tannin in the seeds, but are responsible for the characteristic of the color of the flower. A high correlation is between the color of the flower and the concentration of the other one, as the concentration of the low-concentration tannin is related to the color of the white flower (Grosjean, 2000; Aguilar, 2012).

Table 5 : Represents the results of the primers for the Tannins for Parents and hybrid

N	Primer name	Molecular Wait	Type band	Zero tannins Parents	Zero tannins hybrids
1	OPC -05	551bp	Present	(P2) All parents except	All hybrids except hybrids) 25 , 36(
2	OPG- 11	1171bp	Absent	(P1,P8) Parents	hybrids(13,16,18,24,48,58)
3	OPG -15	600bp	Present	(P1,P6) Parents	hybrids(16,24,68)
4	OPQ -15	476bp	Present	(P1,P2,P4,P5,P7,P8) Parents	(23,25,45,47,48) Hybrids except hybrids
5	OPJ -12	886bp	Absent	Parents(P1,P3,P6,P8)	hybrids(13,18,23,24,67,58)
6	OPJ - 14	504 bp	Absent	Parents(P1,P7,P8)	hybrids(18,24,78,35,37,48)

2- Molecular prediction of the existence of the tannins

In this study (6) short primers referred to in Table (2) that have an association with tannin presence or absence based on research conducted by Gutierrez , *et al.* (2007); Gutierrez , *et al.*, (2008) through a molecular size information bands used either coupling or repulsion attraction are used. With tannins concentration. PCR interactions were performed on fathers and camels to find camels that could be low in tannin, and most results were compatible between parents and camels, and they can be summarized as follows**:**

1 **-Primer OPC-05:** The results of this primer were shown on parents and hybrids, depending on the band associated with the second (551 bp). It turns out that all parents and all hybrids are on the recessive gene of the lower Tannins except for the P2 and the two hybrids (25,36) did not contain the recessive gene and thus this father and the two hybrids are highly Tannins as shown in Table (5) and Picture (1,7).

2-The Primer OPG-11: Through the results of the band-linked tannin (1171 bp), the absence of the band is at the lower level of the tannins and the presence of the band on the higher level of the tannins and the stage on the gel electrophoresis. It turns out that the parents that contain the recessive gene with low tannins are the parents P1, P8 and hybrids (13,16,18,24,48,58). As for the rest of the parents for crosses, they contain the recessive gene, so they are high tannins, as shown in the table (5) And the picture (2,8).

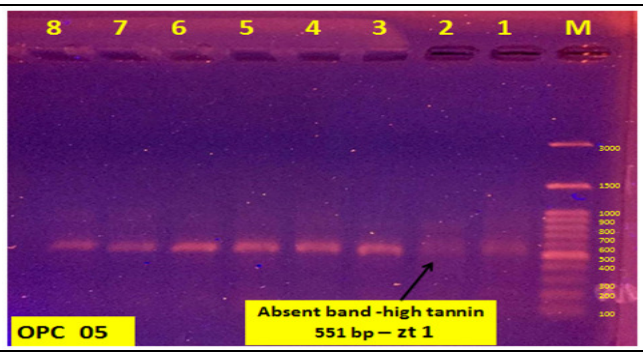
3 -The Primer OPG-15: In the results related to the band (600 bp) and it has a correlation with the recessive gene, the presence of the D band is at the lower level of the tannins and its absence, indicating the high level of the tannins and the stage on the acrylic gel. The fathers that contain a recessive gene with a low-tannins gene are the parents P1, P6 and hybrids (16,24,68). As for the rest of the parents and hybrids do not contain the recessive gene, and thus they are high again, as shown in Table 5 and Image (3,9)**.**

4 -The primer OPQ-15: through the results associated with the band (476 bp) and has a correlation with the recessive gene and the presence of the band is on the lower level of the tannins and the absence of the sign on the high level of the tannins and the stage on the gel electrophoresis. It turns out that the parents that contain the recombinant low-tannin gene are the parents P1, P2, P4, P5, P7, P8 and hybrids (23,25,45,47,48), while the rest of the parents and hybrids do not contain the recessive gene and are highly tannin As shown in Table (5) and Image (4,10).

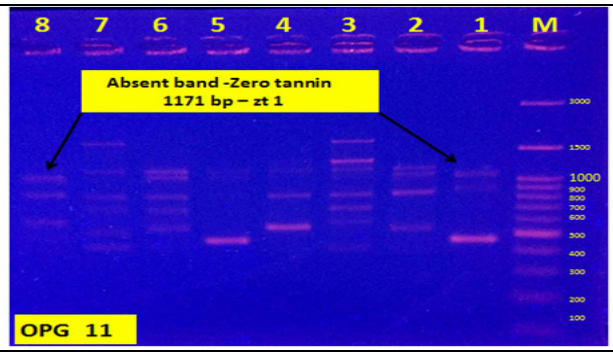
5 -The primer OPJ-12: shows the results associated with the band (886 bp) and has a correlation with the recessive gene recessive and the absence of the band is on the low level of the tannins and its presence is indicative of the high level of the tannins and the stage on the gel electrophoreses. It turns out that the parents that contain the recessive gene are low Tannins are the father P1, P3, P6, P8)) and hybrids (13,18,23,24,67,58), while the rest of the parents and hybrids do not contain the recessive gene and are thus high The tannins, as shown in Table (5) and Image (5,11).

6- The initiator OPJ-14: through the results associated with the band (504 bp) and has a correlation with the mutant recessive gene, and its absence is indicative of the low level of the tannins and the height of the tannins on the height of the tannins level and the stage on the gel. It turns out that the parents that contain the recessive gene with low tannin are the parents (P1, P7, P8) while the rest of the parents and hybrids (18,24,78,35,37,48) do not contain the recessive gene that is highly tannin, as shown In table (5) and in the picture (6,12).

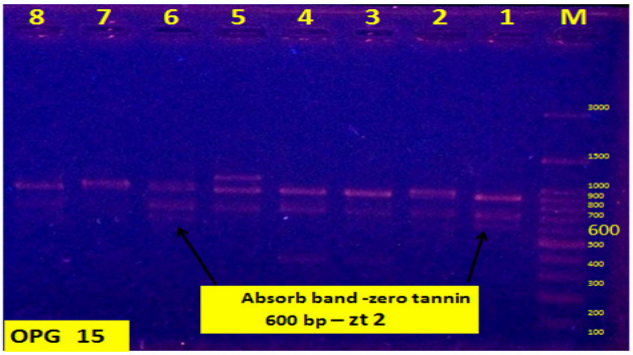
In the totality of the results obtained from the six prefixes used which predicted the concentration of the Tannins in the fathers, it indicated that the parents P1, P8 are among the fathers that are more likely to contain the lower Tannins, while in the crosses the hybrids were the most frequent in the lower Tannins are the hybrids (13,16,18,24,25,48,58).



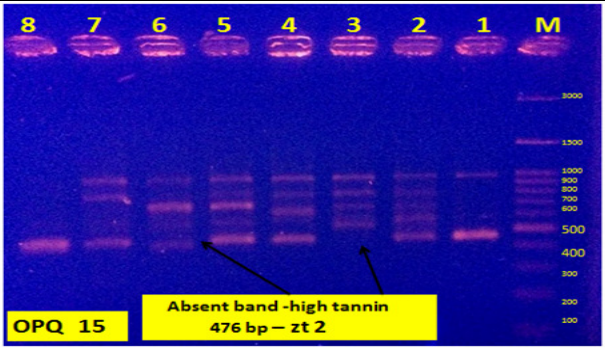
Picture 1 : represents the Primer outcomes of OPC-05 with DNA eighth genotypes as Parents of the *Vicia faba* genus .



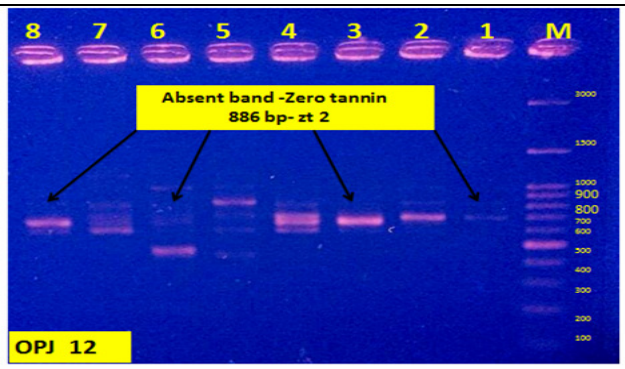
Picture 2 : represents the Primer outcomes of OPG-11 with DNA eighth genotypes as Parents of the *Vicia faba* genus



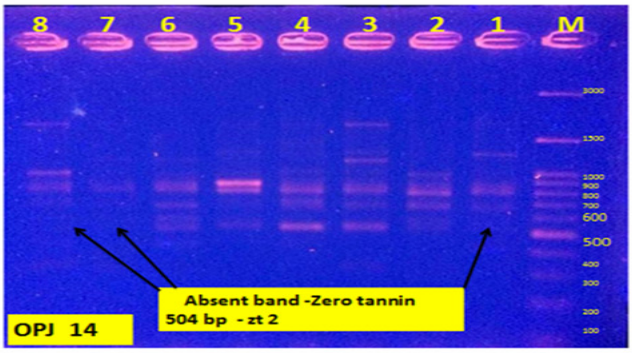
Picture 3: represents the Primer outcomes of OPG-15 with DNA eighth genotypes as Parents of the *Vicia faba* genus .



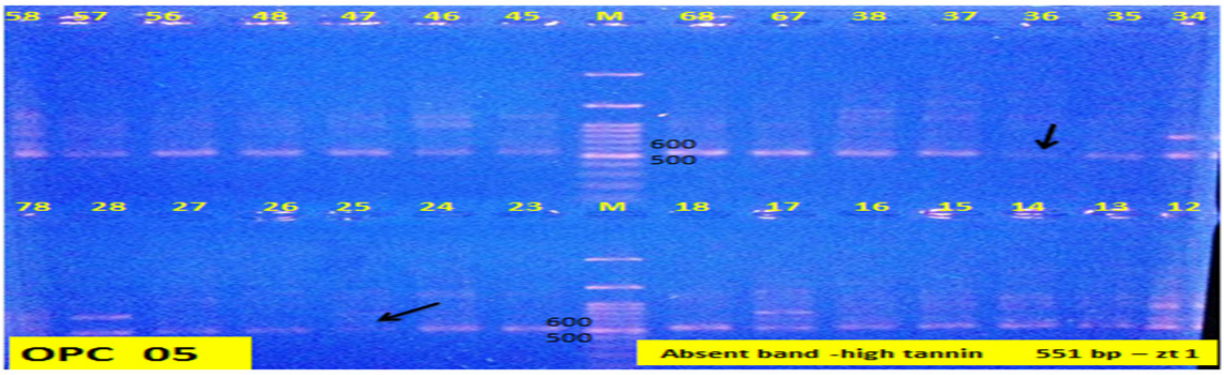
Picture 4 : represents the Primer outcomes of OPQ-15 with DNA eighth genotypes as Parents of the *Vicia faba* genus .



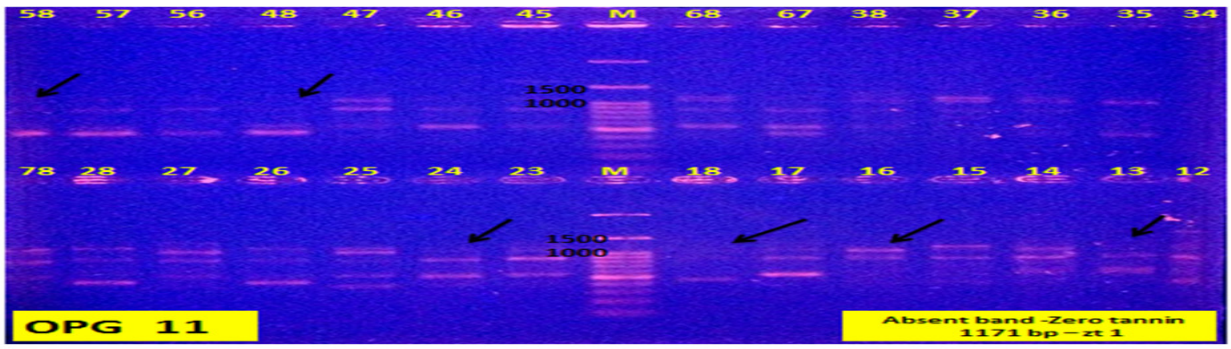
Picture 5 : represents the Primer outcomes of OPJ-12 with DNA eighth genotypes as Parents of the *Vicia faba* genus .



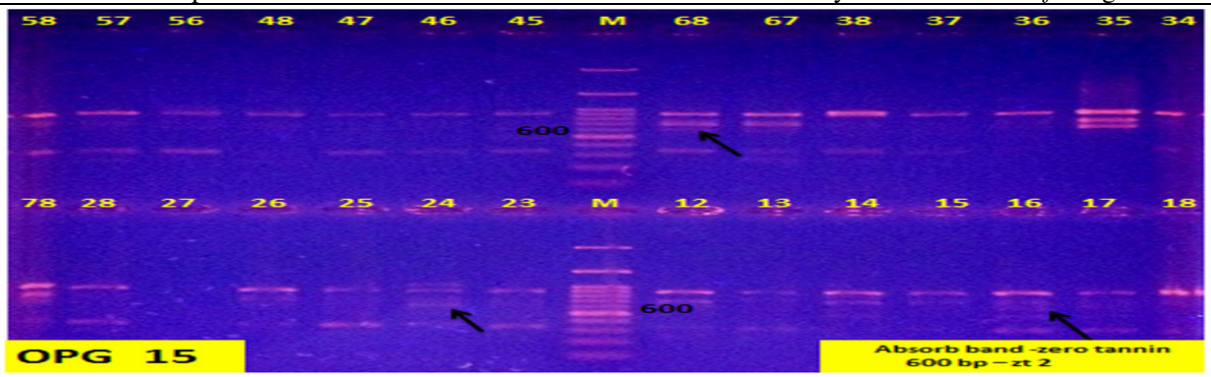
Picture 6 : Represents the Primer outcomes of OPJ-14 with DNA eighth genotypes as Parents of the *Vicia faba* genus .



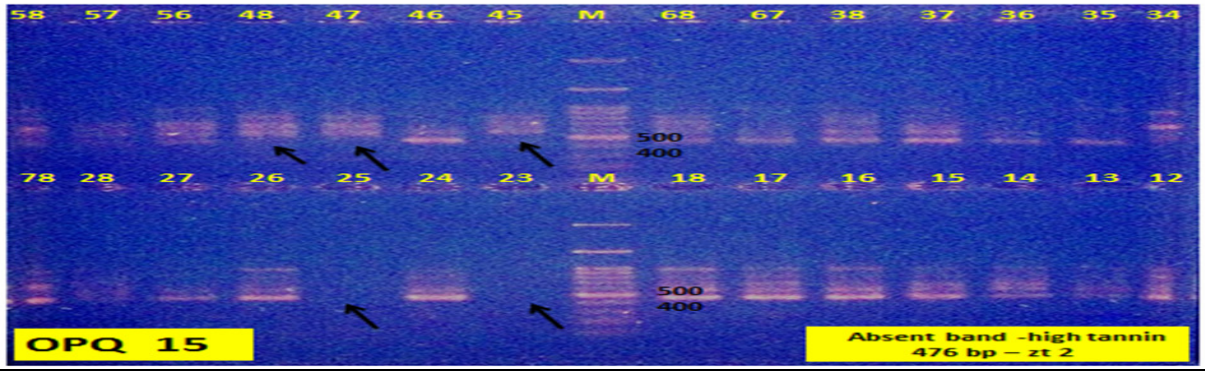
Picture 7 : Represents the Primer outcomes of OPC-05 with DNA 28 Hybrids of the *Vicia faba* genus .



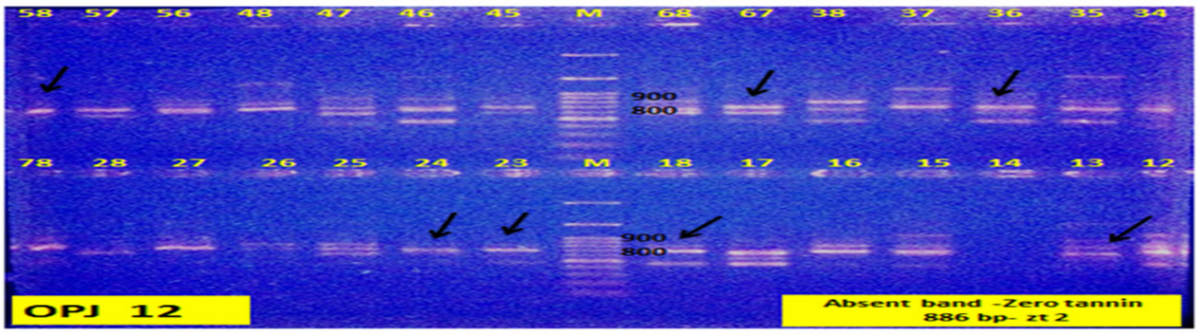
Picture 8 : Represents the Primer outcomes of OPG-11 with DNA 28 Hybrids of the *Vicia faba* genus .



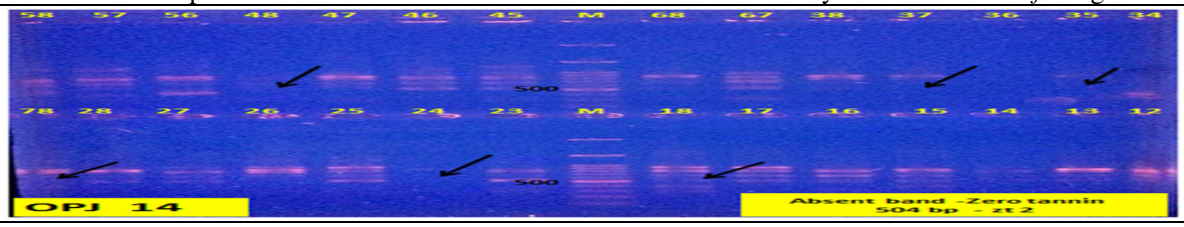
Picture 9 : Represents the Primer outcomes of OPG-15 with DNA 28 Hybrids of the *Vicia faba* genus .



Picture 10 : Represents the Primer outcomes of OPQ-15 with DNA 28 Hybrids of the *Vicia faba* genus .



Picture 11 : Represents the Primer outcomes of OPJ-12 with DNA 28 Hybrids of the *Vicia faba* genus .



Picture 12 : Represents the Primer outcomes of OPJ-14 with DNA 28 Hybrids of the *Vicia faba* genus .

3 -Estimate the second in seeds

After identifying parents and hybrids that are few or low tannins in the early stages of the life of the plant through molecular indicators using special prefixes, tannins in the seeds were estimated after the plant reached maturity and harvest according to the method mentioned (Price *et al.*, 1978; Singh *et al.*, 2012) after drying Seed on the sun's rays, solutions were prepared, experiment steps were performed accurately. More than one sample was taken for each father or hybrid. The experiment was performed. The spectroscopy device was calibrated using a pre-prepared calibration solution, depending on the method presented.

The results presented in Table (6) showed the focus of the tannins on parents, hybrids, and the genius of (8) parents and (28) individual hybrids included in the study, different results between fathers as well as different between hybrids. Through these results, parents and hybrids were divided into three groups depending on the division (Gutierrez *et al.*, 2008), which divided it into two categories, the first category is low-concentration, in which the concentration of the second from CE is 1% or less and high-concentration, in which the concentration of two more than CE is 1%, while the division Ingh *et al.* (2012) has divided it into three categories, the first category of CE is 1% Below and the second category from CE (1% to 1.5%) the third category is higher than CE 1.5%. The results of the first category were the low concentration, in which the concentration of the second from CE was 1% or less, as it reached (3) parents by 37% of the total fathers, while in hybrids it reached (10) hybrids by 35% of the total hybrids, and the second category was medium-focused Confined between CE (1% to 1.5%), which amounted to (3) fathers, at 37% of the total fathers, while in hybrids it reached (11) crosses by 39% of the total crosses, while the third category is highly concentrated, where the concentration is higher 1 5% CE, which amounted to only 26% of parents, while in camel it amounted to 7 crosses with 25% of the total crosses. The percentages of the groups in parents and camels were almost the same, indicating that the adjective Mendelian sovereignty follows, so the lower group in the fathers and camels is considered to be in the ratio of the second and middle classes (3: 1), and this is consistent with what happened to many researchers in the field of the second in the Baqal (Crofton *et al.*, 2000; Gutierrez, *et al.*, 2008; Aguilar, 2012).

The results show that the highest concentration of tannin among fathers is Parent P6 with a concentration of 2,950, while the lowest concentration in father P8 with a concentration of 0,825 and the rest of the fathers ranged between those values, while in hybrids the highest concentration in hybrid 24 was 2,890 and the lowest concentration in hybrid (48) was 0,860 The rest of the hybrids ranged between these values as shown in Table 6**.**

Table 6 : Represents the results of the concentration of tannin in the fathers and camel seeds measured in C.E**%.**

Parents	Tannin concentration C.E.%	Hybrids	Tannin concentration C.E.%	hybrids	Tannin concentration C.E.%	hybrids	Tannin concentration C.E.%	hybrids	Tannin concentration C.E.%
P1	0.975	12	1.330	24	2.890	37	0.990	58	0.884
P2	2.14	13	0.923	25	1.350	38	1.555	67	1.240
P3	1.105	14	1,225	26	0.850	45	1.885	68	1.840
P4	1.425	15	1.115	27	1.150	46	1.325	78	1.260
P5	1.216	16	1.465	28	0.976	47	1.730		
P6	2.950	17	0.875	34	0.935	48	0.680		
P7	0.985	18	0.725	35	0.973	56	1.120		
P8	0.825	23	1.754	36	1.150	57	1.510		

* Low tannin *, Medium tannin *, High tannin

4 -The relationship of the RAPD markers with the concentration of the tannins

The process of making a comparison between the results of molecular markers and estimating the tannins one is a focal point in this project in order to determine the accuracy of the molecular markers in the future prediction of the concentration of tannin, which does not need to reach the plant to the stage of maturity only to obtain a small vegetative part and because it saves time, effort, costs of cultivation and multiplicity Generations (Brake *et al.*, 2014; Dumireih *et al.*, 2010). It is clear from the comparison procedure between the molecular indicators and the percentage of tannin in the seeds was 85% compatible, and this is a good percentage for partial prediction of the tannin, and the reason is due to the lack of reaching the ratio to 100%, which does not mean the inaccuracy of the molecular results, but this ratio cannot be obtained in the leguminous plant Because it contains a high mixing ratio, as well as the occurrence of pollination by insects and winds, as well as that the crossbreeding process is 75% because the plant is self-pollinated (GÖL 2015; Handi *et al.*, 2013; Khan *et al.*, 2010).

It appears from the results presented in Table (5) that the best primers used in identifying the lower tannins in the fathers are the primer OPJ-14 because it corresponds to the estimate of the tannins in the parents at 100%. parents identified P1, P7, and P8. The efficiency of the OPG-11 was high in identifying parents, as it identified the parents P1 and P8, and other primers were continued in the diagnosis of parents. As for hybridization, the primer OPG-11 (4) crossed from the lower two camels out of (6) hybrids and one mean the tannins It is (13,16,18,24,48,58) as well as the initiator OPJ-14 people (4) hybrids of low-tannins crosses and hybrid of tannins medium and high-tannins hybrids e. (18,24,78,35,37,48) as well as the primer OPJ-12 person (3) hybrid low tannin and high tannin hybrid and medium tannin hybrid (13,18,23,24,67,58) as well as other primer s rolled in the diagnosis**.**

Conclusion

The results based on three categories can be analyzed for the concentration of the tannins, namely that the low-tannin group contains the pure mutant genes, which abolished the role of the gene responsible for the wild (high-altitude) gene, and the medium-middle category is in the hybrid state, i.e. it contains a wild gene and the last mutant, which helped to Reducing the concentration of tannin in half. As for the high-tannin model, it contains a pure wild gene, which leads to raising the concentration of tannin (Hormaza, *et al.*, 1998). That the molecular diagnosis based on the RAPD markers gives a positive diagnosis to the parasitic model on the parents and hybrids of both the lower and middle categories of the tannins to the presence of the mutant gene in both, that the occurrence of differences in the modeled expression between the two categories (low and medium) have the environment and plant physiology have a role in changing the focus of the tannins and also the relationship of those The adjective with the other characteristics of the plant (Khierallah *et al.*, 2014; Sanz *et al.*, 2001).

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