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Molecular detection of the flowering gene ZmCCT9 gene appearance in six maize varieties using PCR and fluorescence capillary gel electrophoresis techniques with different flowering times

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ABSTRACT

This experiment was conducted at the laboratories of Al-Musayyib Bridge Company for Molecular Analysis in 2024 to investigate the appearance or non-appearance of the flowering gene among six maize varieties. Four of these varieties are Egyptian, (coded as TW-78, TW-345, IY-355, and IY-207), and two are local (Baghdad-3 and Al-Maha). In this study, we used DNA markers based on PCR (Polymerase Chain Reaction) and electrophoresis techniques, where forward and reverse primers for the flowering gene were utilized to distinguish between varieties in terms of flowering time, whether early or late flowering, based on the appearance or non-appearance of the flowering gene. The results indicated that the primer pairs used with the target flowering gene are highly efficient in diagnosing the genetic performance among the selected varieties based on the differences in their flowering times, using PCR (polymerase chain reaction) and gel electrophoresis techniques. The results indicate that: the Egyptian variety IY-355 did not show any band in the PCR reaction for the flowering gene, evidence that this variety does not possess the target gene. Consequently, it may flower over a wide thermal range (long days). Genes or genetic loci may influence the trait of early or delayed flowering. Further studies are needed. Egyptian varieties (TW-78, TW-345 and IT-207) and local varieties (Baghdad 3 and Al-Maha) showed a unique single band with a molecular weight of 400 bp, indicating the recognition of the forward and reverse primers for the flowering gene on similar sequences in the DNA of the varieties and considering it a genetic fingerprint to distinguish between them. This will assist in developing superior varieties to increase grain yield and achieve self-sufficiency. As well as developing the genotype and expanding the genetic base of the maize corn crop. So to benefit from those results in selecting and determining the most suitable and appropriate method in programs for cultivating maize to obtain genotype with high productivity and promising traits.

Keywords: PCR, Flowering times, Maize, Genotypes.

Introduction

The maize crop is a model for studying genetic organization due to its high genetic and phenotypic variability, which helps in choosing the appropriate breeding method and having a broad genetic base that is available for testing (Al-Amiri, 2021). Early flowering for maize varieties has many benefits, both for grain yield and for the subsequent agricultural processes of planting other crops. Early flowering leads to saving time and effort and the possibility of

utilizing the land for planting other crops. On the other hand, the relationship between early silking and delayed physiological maturity (the length of time between silking and physiological maturity) ultimately results in the longest period for grain-filling, which represents a crucial and important stage in the life of the maize crop. As it is responsible for producing long ears with full and healthy grains, thus increasing the yield (Al Hadi et al., 2013). The maize crop is a monoecious flowering plant, and the flowering process begins when temperatures rise. In Iraq, flowering occurs in May when planted in the spring season, while in the fall season, flowering takes place in August-September. Baktash and Mazall have found. (1985) There is a negative correlation between grain yield and maximum temperatures during flowering times and fertilization in the spring and autumn seasons. Flowering is the stage where vegetative growth ceases as plants begin their reproductive growth. It is one of the most influential growth factors, alongside light intensity, temperature, soil moisture, and their interaction with the crop's genetic structure (Alousi, 2005). Meanwhile, the relationship during the same period was positive with relative humidity in the atmosphere during the growing season. The appearance of reproductive parts represented by tasseling and silking inflorescences and the production of pollen in maize is one of the most important and sensitive stages for the growth and development of the crop, particularly concerning the final grain yield. Through the information obtained about understanding the nature of genetic actions and identifying their interactions in the transmission and inheritance of traits and flowering in different varieties, it is used in cases where the target gene is present in the quantitative traits of the crop, such as the ZmCCT9 gene responsible for the flowering process (Huang et al., 2017). The flowering time is one of the critical factors for the adaptation of crops to local environments. It is sensitive to low light periods and adapts to areas within 90 degrees of latitude, especially in the Americas, ranging from 58 degrees north to 40 degrees south. Here, the need for the flowering gene ZmCCT9 has emerged, which contains regulatory elements like Harbinger-like transposable elements that work to reduce its gene expression under long-day conditions, thus helping the maize crop to flower across a wide range of environmental conditions, particularly temperature and photoperiod factors. Many studies have indicated that Cis-action variables typically enhance the expression of the ZmCCT9 gene to promote early flowering under long-day conditions (Hung et al., 2012; Salvi et al., 2007). Because of natural and artificial selection and hybridization, varieties of maize have been developed for different thermal ranges around the world, with early and late varieties found in their flowering times. There are more than 462 gene sites related to quantitative trait loci (QTL) that have been identified in maize. (Buckler et al., 2009), which significantly contribute to phenotypic variation, and their effect is of the additive gene action type. Xie and others (2010) indicated that there are approximately sixteen QTL sites controlling seven traits of tasseling and silking in maize. Four QTL sites are responsible for the duration between male and female flowering, known as an thesis Silking Interval (ASI); seven QTL sites are responsible for tasseling flowering and pollen release; and five QTL sites are responsible for the number of days to silking flowering, silk emergence, and pollen reception. Many studies have shown that late-flowering varieties have an allele that significantly affects twenty-four QTL sites, with 75% responsible for the delay in flowering. Meanwhile, early flowering inbreed lines and varieties have an allele that significantly affects eighteen QTL sites, with 66% responsible for the early flowering (Buckler et al., 2009).

Because of the discovery of modern technologies, which indicate the beginning of scientific investigation and detection of the flowering trait, especially the molecular indicators based on DNA, there is an increase in knowledge regarding the interpretation between the flowering time trait and interacts with environmental surrounding conditions during the growing season. This allows for the identity genes responsible for the trait inheritance and understanding of their functioning under various prevailing environmental conditions during the growing season, forming what is known as effective genomic tools (the variation in gene expression depending on the nature of the gene and the genetic structure, whether it is a variety, hybrid, or cultivar of the crop). Among these indicators are DNA indicators that rely on the DNA of the organism. An important feature compared to phenotypic indicators, as they are not affected by surrounding environmental conditions and instead depend on the organism’s genetic material, the DNA, distinguishes them. It is used to detect the genetic material DNA carried by the species as well as to identify the genetic variations among them. Among these technologies is the PCR technique, which facilitates the processes of hybridization and selection for plant breeders in the quest to find and develop new hybrids and maize

varieties of high yield. The PCR technique is considered one of the older technologies theoretically and the most commonly used practically, having been discovered by scientist Kary Mullis in 1985. It is an extracellular enzymatic reaction through which short sequences of DNA are amplified and duplicated using polymerase enzymes, nitrogenous bases, and specialized or random templates (Al-Hadeithi, 2012).

It is a practical technology based on amplifying copies of DNA outside the cell and controlling the DNA quantity and the speed of production. One of its disadvantages is the lack of a repair system when the wrong connection occurs. The technology principle is based on amplifying a specific piece of DNA from the entire genome. Enzymatically outside the living organism using a thermal cycler and in the presence of primers, which bind to the complementary sequence with the DNA template strand due to the DNA action of the polymerase enzyme, along with a buffer solution and nitrogenous bases to obtain large quantities of the desired DNA amplified (Kumar et al., 2009; Al-Hadeithi, 2012).

The current study aims to detect and confirm molecularly, using molecular indicators at the DNA level, the presence of the flowering gene among six varieties of maize, four of which are Egyptian (carrying the codes TW-78, TW-345, IY-355, and IY-207) and two locals (Baghdad-3 and Al-Maha) with different flowering dates using PCR and electrophoresis techniques to determine genetic changes between the studied varieties. In order to determine the best ones on them, and to identify the flowering gene and use it in building genetic maps to develop the genetic structure and expand the genetic base of the crop. And benefit from these results to determine the best varieties to be included in breeding programs to obtain the desired and promising genetic compositions (varieties).

Materials and Methods

Molecular experience: The laboratory experiment was conducted at the Al-Musayyib Bridge Molecular Analysis Laboratories in Baghdad Iraq in 2024. To study the appearance or nan- appearance flowering gene among six maize varieties, four of which are Egyptian, bearing the codes (TW-78, TW-345, IY-355, and IY-207) from Dr. Omar Muhammad Zboun of Al-Azhar University, Arab Republic, Egypt, and two local varieties (Baghdad-3 and Al-Maha) from the Agricultural Research Department, ministry of Agriculture Iraq with different flowering times, as shown in Table (1). The DNA total each variety was extracted using Cety Trim ethyl Ammonium Bromide (CTAB) as mentioned by (Weigand et al., 1993). After verifying the quantity, the DNA extracted and performing the appropriate dilution process, the DNA extracted doubled using forward and reverse primer pairs with identical sequences. A short sequence within the PCR technique based on the appearance or non-appearance of DNA duplicated fragments and their location on the gel electrophoresis.

Samples varieties used in the study (Genotype): The laboratory experiment was carried out in the laboratories of Al-Musayyab Bridge for Molecular Analysis Company, Baghdad - Iraq, for the year 2024. Six maize varieties used in the study, as shown in Table (1). The ears placed in special bags in the Cooling Box to maintain the quality of the samples until they transported to the laboratory to DNA extracted from them.

DNA isolation: DNA extracted from the mature seeds of the studied varieties in the laboratories of Al-Musayyab Bridge Company in the year 2024, using CTAB, as mentioned by (Weigand et al., 1993). As this method included obtaining 200 micrograms per 2 g of mature seeds (representing the samples) for each studied maize variety crop. The DNA purity values ranged between (1.7-1.9).

Extracting DNA from the studied samples: This method includes CTAB as described by (Weigand et al., 1993) .

1. 1.200 milligrams of seeds from each sample of the six studied varieties are ground after sterilizing those using sterilizers, then adding liquid nitrogen to a ceramic mortar containing 200 milligrams of sample until the seeds are completely ground and white powder obtained as fine as possible.
2. Then put the white powder in 1.5 ml test tubes and add 12 micro liters of CTAB extraction solution. The tubes are placed in a water bath for one hour at a temperature of 55 Celsius.
3. Then remove the tubes from the water bath and leave them in the room temperature gain.
4. Add 400 microliters of chloroform to the tubes، stirring continuously using the Vortex device for 15 minutes.
5. Then transfer the tubes to the centrifuge at a speed of 4000 rpm for 15 minutes.

Table (1): Names and symbols of maize varieties used in the study, their source, and their tasseling and silking dates.

Varity number	Variety symbol	Number of days from planting to 75% tasseling (day)	Number of days from planting to 75% silking (day)	Pedigree of varieties
1	Baghdad 3	49.50 early	53.50 early	Ministry of agriculture department of agricultural research
2	Al-maha	50.75 early	55.12 early	Ministry of agriculture department of agricultural research
3	TW-78	47.75 too early	52.62 too early	Dr. Muhammad Omar Zaidon Al-Azhar Egypt university
4	TW-345	53.88 late	57.50 late	Dr. Muhammad Omar Zaidon Al-Azhar Egypt university
5	IY-355	55.38 too late	59.25 too late	Dr. Muhammad Omar Zaidon Al-Azhar Egypt university
6	IY-207	47.88 too early	53.75 too early	Dr. Muhammad Omar Zaidon Al-Azhar Egypt university

6. Remove the upper aqueous layer (the filtrate formed after the centrifugation process) in the previous step using 1 ml pipette tubes.
7. Step No. (4) is repeated.
8. Then remove the upper aqueous layer (filtrate) formed after the centrifugation process in the previous step using pipette tubes with capacity 1 ml ، put it in new tubes with capacity1.5 ml and add 5 ml of cooled iso- ethanol.
9. The tubes mixed and stirred continuously until a white mass appears ، representing the formed DNA strands (DNA extracted) from the seed samples of the six studied varieties.
10. Then the DNA extracted, which is in the form of white threads is pulled out using a hook-shaped tube, and placed in new test tubes with a capacity of 1.5 ml, 400 microliters of washing solution, and the care tubes for 20 minutes.
11 .Repeat the previous step by transferring the DNA strands into new 1.5 ml tubes containing (200-400) microliters of TE solution (dissolution and stirring solution) from time to time until the melting process is complete.
12. Preserve the DNA extracted of the seed samples of the selected varieties in Deep Freezing at a temperature of (-20) Celsius until later use

Estimation of DNA concentration and purity: The assessment of the purity of the DNA extracted from the previous steps is to ensure that the DNA extracted is pure and of good quality. The DNA obtained from the previous section (DNA extraction process) is diluted by adding 990 microliters of distilled water to 10 microliters of the DNA extracted, due to the presence of large amounts of polysaccharides and phenolic compounds in plants, which precipitate with the DNA, forming a viscous liquid. Inhibitory substances are present that hinder the PCR technique, so the DNA extracted is diluted to reduce those inhibitory materials that impede the occurrence of the PCR reaction. The amount of absorption of the sample for ultraviolet rays is read using a device called a UV spectrophotometer, which relies on measuring the sample's absorption of ultraviolet light at a wavelength of 260 nanometers and at a wavelength of 280 nanometers (Maniatis et al., 2001). The concentration is calculated using the following equation:

DNA purity = 260/280: 1.7-1.9
the concentration of DNA in micrograms per microliter equals the absorbance at a wavelength of 260 multiplied by 50.

Nanometer/ml of the diluted sample × dilution factor × 40.

Purity = absorbance at a wavelength of 260 nanometers/absorbance at a wavelength of 280 nanometers, and the reading is recorded:
The purity ratio is one of the indicators of the purity of DNA extraction and the extent of its contamination with protein.

DNA purity = 260 O.D. / O.D. 280≤ 1.8
The division result should be greater than or equal to 1.8. If the division result is lower, this indicates that the DNA sample is impure and requires a new extraction of DNA. The results of the purity concentrations are shown in Table (2).

Table (2): Concentrations and of purity degree of DNA extracted from selected varieties

ID sample	Abs260	Abs280	260/280	Con Smple ng/ul
Baghdad 3	0.952	0.516	1.84	34 ds DNA
Al-Maha	0.988	0.558	1.79	23.2 ds DNA
TW-78	1.904	1.019	1.87	13.4 ds DNA
TW-345	1.254	0.635	1.97	15.1 ds DNA
IY-355	1.318	0.797	1.65	26.8 ds DNA
IY-207	1.443	0.796	1.81	30 ds DNA

PCR Reaction Steps:

The importance of using forward and reverse primers for the PCR reaction: In this study, I used two forward and reverse beginnings obtained from BIONER KOREA, as shown in Table No. 3. The importance of using of both the forward and the reverse primers in the PCR reaction is to make the reaction more specific and specific to the target gene. To achieve this goal, two things must be achieved: the length of the starter and the actual sequence of the starter must be sufficient to ensure that detected by the target gene (the flowering gene in this study).

Table (3): Shows the lists names of the two primers used and their sequences used to identify the flowering gene using PCR technique

Name primer	5"-3" sequence primer
Forward primer *ZMCCT9* gene responsible for flowering	5"TCAAAGAAGATAAAGAGCGGAG "3
Reverse primer *ZMCCT9* gene responsible for flowering	5" ACGCCCTCGTTAGGTTCATA"3

Molecular detection of the flowering gene: For the purpose of molecular detection of the flowering gene *ZmCCT9* gene, polymerase chain reaction and gel electrophoresis techniques were applied.

First: detection using PCR technology: The PCR technique was applied using nucleic acids extracted from maize studied samples using the previous method and based on the forward and reverse primers of the flowering gene, as shown in Table (3).

DNA fragments duplicated extracted from the six selected varieties using a PCR reaction program: (Steps of the PCR reaction program)

Follow the working method attached to the kit prepared by the Korean company Pioneer Accu power PCR premix:

1. Add 13 microliters of distilled water
2. Add 1 microliter of the forward and reverse primers of the flowering gene.
3. Add 1.5 microliters of magnesium chloride at a concentration of 25 microliters to the reaction tube.
4. Add 5 microliters of the DNA extracted sample specific to each genetic structure to a tube
5. Add DNTPs at an appropriate concentration to the reaction tube
6. Add Taq DNA Polymerase at an appropriate concentration to the reaction tube
7. The total volume becomes 20.5 microliters with the material already present in the tube.
8. Shake the contents in the reaction tube well and leave for two minutes until the additives are homogeneous.
9. The samples are placed in the thermal polymerization device and the following program is executed as shown in Table (4)

The steps of the thermal program:

10. Initial denaturation first cycle at 95°C for five minutes.
11. A second denaturation second cycle at a 95°C for 30 seconds and forty cycles.
12. The docking of the flowering gene takes place at a temperature of 57°C ،and forty- one minutes of the cycle.
13. The initial elongation of the flowering gene occurs at a temperature of 72°C and for forty minutes a cycle that repeats the steps from (repeat steps 7-11).
14. The second elongation of the flowering gene takes place at a temperature of 72°C ،for one minute، and the cycle one.
15. Chilling step at a temperature of 4 Celsius.

The steps of the thermal program: This complete according to the steps shown in Table (4).

Table (4): The steps of the thermal program

No.	Steps	Temperature	Time	No. cycle
1	Initial Denaturation	95°C	5Min	1
2	Denaturation	95°C	30Se	40
3	Annealing for flowering gene °C57	57°C	1 Min	
4	First extension (repeat steps from 2-4 steps (40 cycle)	72°C	1 Min	
5	Second extension	72°C	1 Min	1
6	Chilling	4°C		1

After completing the reaction, the samples placed in the freezer (kept at low temperatures) until use for electrophoresis (agarose gel).

Electrophoresis of the PCR product on an agarose gel:

Prepare agarose gel: The electrophoresis process of the DNA products replication process was carried out on agarose medium at a concentration of 2%, which prepared by following these steps:

1. Dissolve 2 g of gel powder in 100 µl of X1 TBE solution.
2. Heat the mixture using the microwave at a temperature of 50-60 Celsius for two minutes, stirring from time to time, until the mixture is melted and homogeneous well.
3. Then pour the gel solution into the tray prepared for electrophoresis، which in turn contains the fixing comb that is surrounded by adhesive tape، to later the injecting (loading pit). The DNA extracted from the studied samples.
4. Pour the gel solution continuously and gently to prevent bubbles from occurring if they form, they must remove using special tubes prepared for this purpose.
5. Leave the gel solution at room temperature to harden and as the gel hardens, the comb lifted and immersed in quantity a sufficient of X1 TBE solution. To cover the loading injection holes in the basin Tray.

PCR products were migrated onto an agarose gel: After preparing the agarose gel intended for migration of PCR duplicated products, the following steps are followed:

1. This is done by mixing 3-5 microliters of the DNA sample and the PCR products of the six selected species with (2-3) microliters of loading buffer.
2. The standard indicator of the DNA ladder is injected into the left end of the gel of different lengths Figure (2). Use a length of (100-2000 Pb) into the first hole of the gel for comparison in our study, which in turn represents the standard sample. After injecting the DNA standard indicator, the samples are simply loaded into the gel pits the sequence marked in the plan.
3. Then perform the electrical relay after operating the power supply for 54 minutes amount of 5 volts/cm، until the nearest edge of the agarose gel arrived.
4. Then lift the gel and place it in a basin containing Ethidium bromide dye at a concentration of 0.5 micrograms in one place dark for half an hour.
5. After the transfer process is completed، the mold is transferred to examine the gel by exposing it to an Ultra Violet light source.
6. Photographing and documenting the results of electrophoresis of samples photographically.

The results analysis of molecular study included converting the results, that appear in the gel into tables and the bands resulting from process were calculated by: making number Symbolize the band appearance take number 1 and the number 0 indicate the band nan- appearance. The molecular size of DNA replication products estimated and compared to guide DNA Ladder volume.

Results and Discussion

DNA Isolation from the studied varieties: DNA was extracted from the seeds of the six maize varieties used in the study according to the CTAB method as described by Weigand et al. (1993). The DNA extracted using this method is suitable for PCR reactions in terms of quantity and purity. A good amount of DNA extracted was obtained, estimated at (150–200) micrograms per 20 grams of seeds for each variety, with a purity ranging between (1.7 - 1.9) . The absorption of ultraviolet light at wavelengths of 260 and 280 nanometers was relied upon to estimate the quantity and purity of the extracted DNA. The DNA samples were diluted to achieve a concentration of 50 Nano-grams per microliter, which is the appropriate concentration for conducting PCR reactions.

Molecular analysis of the forward and reverse primer pairs used in identifying the flowering gene loci for the six maize studied varieties: To analyze the molecular state behavior of studied varieties based on their flowering times. through the appearance or nan –appearance of flowering gene in the selected varieties, to utilize those results in choosing suitable

programs for the breeding of maize After completing the electrophoretic transfer of the DNA amplified samples using PCR technology and staining them with red dye, images are captured using ultraviolet light. The method of analyzing and studying genetic variation was based on the DNA extracted from the selected varieties, relying on the principle of band appearance in the gel. The reason for the appearance of the band (size bp400) in most of the selected varieties is evidence of the presence of the gene in the genetic structures used in the study (Variety 1, Variety 2, Variety 3, Variety 4, and Variety 6). This is due to the ability of the forward and reverse primer pairs to locate their complementary sites within the DNA sequences of the selected varieties. As for variety number (5), the nan- appearance band because the variety does not carry the target gene (flowering gene), as shown in figure (1).

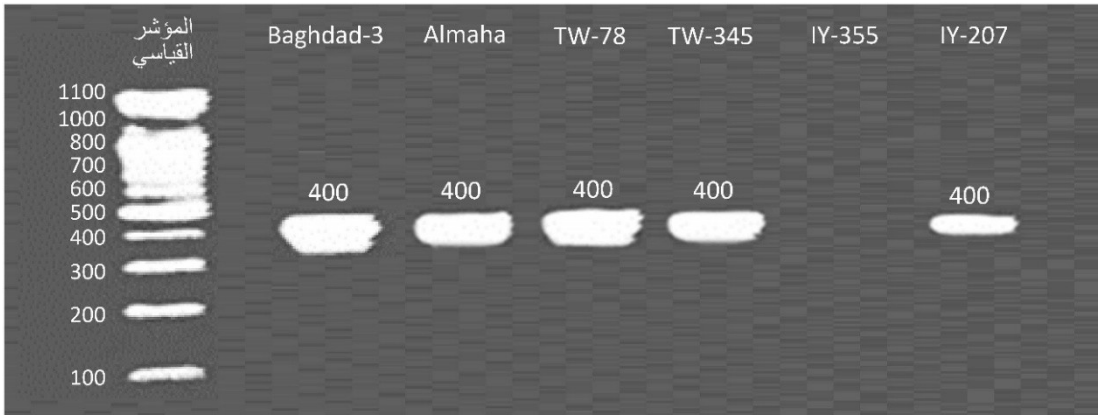

Figure (1): It illustrates the results of agarose gel electrophoresis for identifying the flowering gene locations in studied varieties of maize

Agarose gel electrophoresis analysis of PCR products to identify the locations of the flowering gene in the first variety (Baghdad 3) early flowering of the maize crop photographed under ultraviolet rays after staining with Ethidium bromide dye:

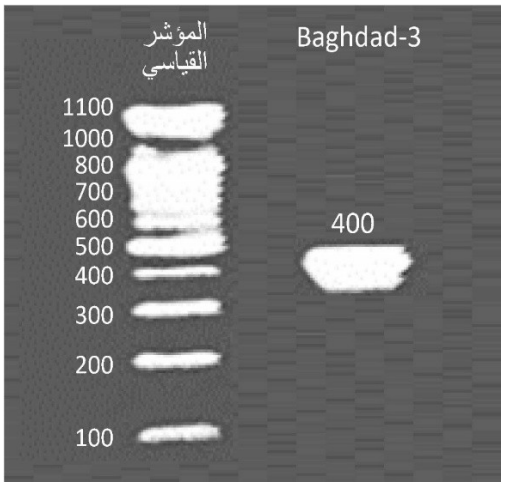

Figure (2): Analysis of results of agarose gel electrophoresis of DNA products replicated using PCR technique with the forward and reverse primers the flowering gene for the Baghdad 3 early flowering variety of maize crop on 2% agarose gel.

The results of the electrophoresis analysis of the PCR products of the first early-flowering variety, as shown in Figure (2). that single bands obtained for the early-flowering variety Baghdad 3 was evidence that the two primers were able to recognize their complementary sequences in the DNA of the Baghdad 3 variety. Thus, the genetic structure of the Baghdad 3 variety is can be distinguished from the rest of the studied varieties genetic structures.

Analysis of the results of agarose gel electrophoresis of DNA products replicated using PCR technique using a pair of primers with Al-Maha early flowering variety of maize on a 2% agarose gel: These two primers provided a single band for the early flowering variety (2) AL-Maha, as shown in Figure (3). These primers demonstrated their ability to recognize the complementary sequences in the DNA of the variety within specific flowering ranges (very late flowering does not show the action of the flowering gene). In addition, another evidence that there is a compatibility between the initiators and the sequence of the template DNA.

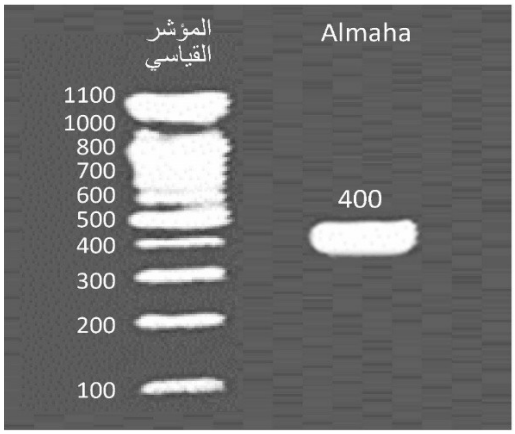

Figure (3): Analysis of the results of electrophesis of PCR-amplified DNA samples using a of primer pairs with the flowering gene of early flowering variety number (2) Al-Maha of maize on a 2% agarose gel.

Agarose gel electrophoretic migration on agarose gel of PCR products to identify the flowering gene sites of the very early flowering Egyptian variety (TW-78) of maize, photographed under ultraviolet light after staining with Ethidium bromide: The results of the electrophoretic analysis for the Egyptian number three (TW-78) show that they had a single band for the variety, indicating their ability to recognize the complementary sequences in the DNA (evidence of the presence of complements for them in this type), as shown in Figure (4).

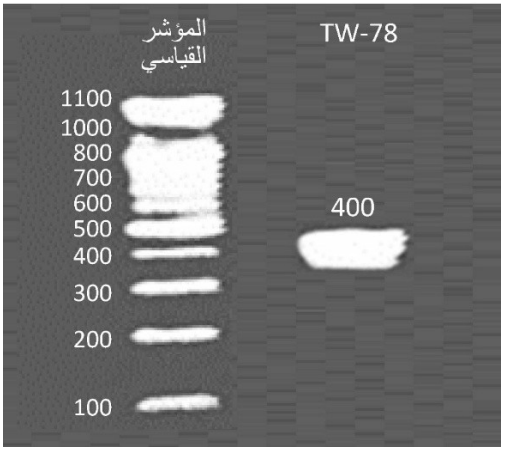

Figure (4): analysis of Results the electrophoresis of DNA amplified samples using PCR technology with forward and reverse primer pairs, along with the flowering gene for the very early flowering Egyptian variety (TW-78) of maize on a 2% agarose gel

Analysis of the results of agarose gel to identify the flowering gene loci for the late-flowering Egyptian variety number 4 (TW-345) of maize: The results of the agarose gel electrophesis analysis of the late-flowering Egyptian variety number (4) (TW-345), showed that these two primers were able to obtain a band for the late-flowering Egyptian variety number (4) (TW-345), indicating compatibility with it. This proof that variety can be distinguished from other varieties (there is compatibility between the DNA sequences of the variety, resulting in a fragment with a molecular weight of 400 bp, as shown in Figure (5).

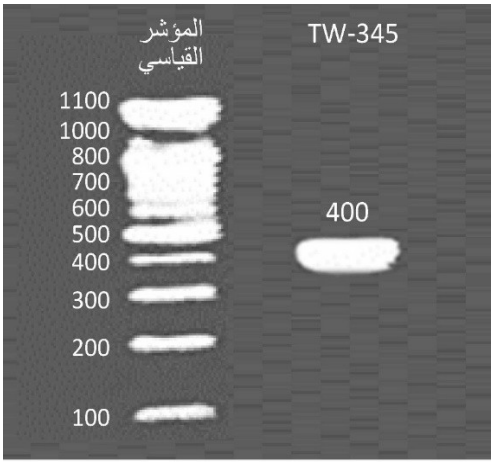

Figure (5): Analysis of the results of electrophesis of pcr amplified DNA samples using primer pairs with flowering gene of the late-flowering Egyptian variety number 4 (TW-345) for maize on a 2% agarose gel.

Analysis of results of agarose gel electrophoresis of DNA products replicated using PCR technique using a primer pairs with the Egyptian variety (IY-355) number 5, which is very late in flowering for the maize crop: The results of the agarose gel electrophoresis for the Egyptian variety number (5) delayed in flowering (TW-345) and nan-appearance band shown in Figure No. 6. For this variety, there was no compatibility between the primer sequence and the DNA sequence, resulting in the absence of a fragment with a molecular weight of (400 BP) (the primer pairs did not find a sequence for it in the genome of the mentioned variety, thus genetic fingerprint for the above variety).

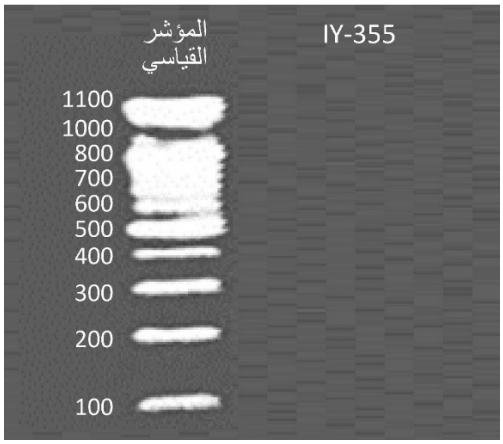

Figure (6): Analysis of the results of the electrophoretic of PCR-amplified DNA samples using forward and reverse primers with the flowering gene of the Egyptian variety (IY-355), which is very late in flowering, of maize crop on a 2% agarose gel.

Analysis of the results of electrophoresis for PCR products to identify the flowering gene sites of the early flowering Egyptian variety (IY-207) of maize, visualized under ultraviolet light after staining with Ethidium bromide: The results of the electrophoresis analysis of the PCR products for the early flowering Egyptian variety (IY-207) showed in Figure (7) that a single band was obtained for the variety, indicating that the primer pairs were able to recognize their complementary sequences in the DNA of the early flowering Egyptian variety (IY-207). Thus, the genetic structure of the variety can be distinguished from the other genetic structures studied varieties (it can be considered a genetic fingerprint for this variety in these two primer pairs). This confirms what was obtained by the researchers Al-Baydani and Hadi (2023) and Mirza and Hadi (2024).

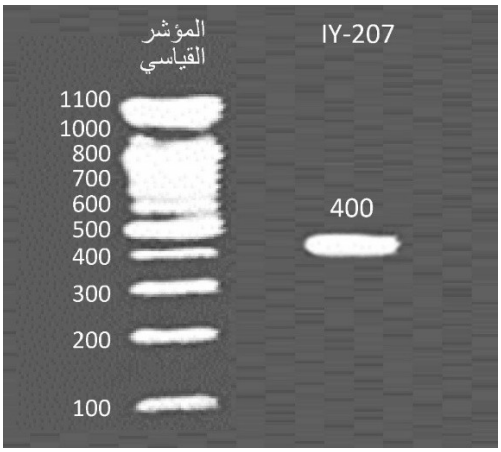

Figure (7): Analysis of the results of electrophoretic of PCR-amplified DNA samples using forward and reverse primers with the flowering gene of the early flowering Egyptian variety (IY-207) of maize on a 2% agarose gel.

The results of the current study indicate that the primer pairs used with the flowering gene can identify the fingerprints of most of the studied varieties. Varieties (Baghdad 3, Al-Maha, TW-78, TW-345, and IY-207) were identified with a package of length bp400. Evidence of the presence of the flowering gene in those genetic structures. While there is no evidence of any band of the IY-355 variety indicating the absence of the genetic structure for the flowering gene due to the influence of another gene or other genetic loci, this requires future studies or the use of different primer structures (varying sequences) with these two techniques to understand this variety and determine its fingerprint. Molecular markers used to detect the appearance of flowering genes that affect the performance of the studied varieties. The absence of flowing gene expression indicates that the variety is not sensitive to photoperiod, allowing for continued flowering across different temperature ranges. This is important for expanding maize cultivation in areas with long daylight hours. On the other hand, the molecular markers allow access to the desired gene loci (the flowering gene) within the closest possible genetic distance (the higher the likelihood of its association with the gene sequence). The studied trait is flowering, which is a quantitative trait controlled by a large number of genes. Therefore, it is essential to consider learning the diversity of the molecular markers of this gene and linking them to its environmental performance, thus aiding in using hybridization processes for early screening of maize variety.

Conclusions

We can conclude from the current study, that the primer pairs used with the flowering gene can determine the fingerprint of most of the varieties, as the varieties (Baghdad 3, Al Maha, TW-78, TW-345, and IY-207) were identified by the band appearance with a length of bp400, evidence of the presence of the flowering gene in those genetic structures. No band of variety (IY-355) appeared to prove that the genetic structure does not have the flowering gene due to the influence of another gene. Genetic sites require further studies or the usage of other primers for the same two techniques and with different sequences to identify this variety and determine its fingerprint. Modern molecular markers technology is

used to confirm the appearance or non-appearance of the flowering gene that affects the performance of the studied varieties. In the absence of gene expression, the variety is not sensitive to the photoperiod; therefore, it can continue to flower over wide thermal ranges, which is important for spreading maize cultivation in areas with long days. On the other hand, molecular indicators allow access to the sites of the required genes (flowering gene) in the closest genetic space possible, the closer the molecular indicator is to the gene, the higher the probability of its association with the flowering time trait is a quantitative trait controlled by a large number of genes. Future studies consider studying the diversity of molecular indicators of these genes linked to their environmental performance and thus help in their use in the hybridization of varieties and early screening time of studied varieties.

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