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The Scientific Effects of Different Sounds Volume on the Phenotypic and Genetic Markers of *Cucumissativus*

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Abstract

The effects of sound on the growth, yield and development of plants have been a subject of scientific discovery. This study aimed to investigate the effects of different volume of sounds of the Holy Qur'an on the growth and development of two varieties of cucumber plant (Aseel and Gazer). It involves the exposure of these two varieties of cucumber to two different frequencies of the Holy Qur'an during the entire period of growth until maturity and evaluating the effects on the phenotypic and genetic characteristics of the plants. The results of the study on the phenotypic traits revealed that the exposure of the (Aseel) variety to high volume and frequency of the Qur'an resulted in the highest number of fruits, leaf length, and plant height compared to the control (same type, with exposure to sound). Similarly, the Gazer cultivar exposed to lower sound volume had the lowest number of leaves, nodes, leaves width and length compared to the control of the same variety. The two varieties were subjected to RAPD-PCR analysis. The results showed that the Aseel cultivar exposed to loud Qur'an sound exhibited 11 unique bands compared to the control group (2 bands), while those exposed to low sound showed 9 bands. It could, therefore, be inferred that exposure to loud sounds of the Qur'an increases the occurrence of mutations. On the other hand, for the Gazer cultivar, the group exposed to the loud sound of the Qur'an showed 14 unique bands more than the control with 3 bands. Furthermore, the group exposed to the low voice of the Qur'an showed 12 unique bands. Our findings revealed that different pitch and volume of sound significantly affect phenotypic markers and cucumber's genome. Increasing the pitch of a sound is shown to have adverse effects on cucumbers' genome due to mutation. The results further revealed that plants show characteristics, feelings and sensation when exposed to different conditions, including sound.

Keywords: *Cucumissativus*, Holy Quran, RAPD-PCR, Phenotypic markers, Genome.

Introduction

The scientific miracle of the Holy Qur'an is the belief that the Qur'anic text expresses universal truths hitherto not known by humans at the time the Qur'an was written. It has now been proven by science in a manner consistent with the belief of Muslims that the Qur'an validates the prophethood of Muhammad bin Abdullah. However, some consider thatthe Qur'an style exhibits a lot of rhetorics. Others believe in the universality of the

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miracle in the Qur'an to be absolute. Hence, Muslims believe that God is the Creator of the universe, and since the Qur'an is his word, it is impossible to contradict the scientific facts that were discovered after the revelation of the Qur'an (Shahwan, 2007). The scientific miracle of the Qur'an means the compatibility of the Qur'anic text with the requirements of modern science or the existence of glimpses or statements within it that confirm scientific facts that were known later (Al-Kheil, 2006).

The Noble Qur'an is the guiding book for leading people towards God Almighty, from whom various methods of inference have benefited. More than one verse was referred to as scientific subjects in the book, which some considered a scientific miracle of the Qur'an. There are verses related to plants and related issues that express the importance of the subject in question. The plants mentioned in the Qur'an attach great importance to their uses and properties (Isfahani, 2004). All the plants mentioned in the Qur'an are sacred and have nutritional and medicinal importance, and have amazing secrets that have been discovered and yet unknown. Since time immomerial humans have paid particular attention to the importance and value of natural resources in their lives.Various verses in the Qur'an encouraged a man to cultivate different plants. In other words, the Qur'an is believed to be the first book to consider the importance of climate information and climate changes in agriculture (Mustansir, 2006).

Some Islamic studies interested in the scientific miracle of the Holy Qur'an indicated that plants, like all other creatures, feel, hear, and respond either negatively or positively to external influences around them. There is no doubt that the Qur'an has a strong effect on the plant. And this prostration is a manifestation of being influenced by the Holy Qur'an, and perhaps this effect which is represented in prostration is not the only aspect, but there are many other manifestations, such as: crying and groaning, and this has been scientifically proven, such as the Pat dry-eyed phenomenon Guttation (Al-Ghali, 1987).

It is stated in the Qur'an about praising the creatures with the praise of God, and the plant is a creature that speaks real words and praises the glory of God (Blkasm, 2007).

Sound affects organisms, including plants, and sound is energy in the form of a vibrating pressure wave transmitted through gases, liquids, and solid materials. It travels through air at a speed of approximately 340 m/s, and for every sound, there is a particular frequency. The human's audible range ranges from 20 vibrations per second to 20,000 vibrations per second (Rokhina*et al*., 2009). In a study, sound waves were applied to okra and zucchini seeds using birds that echo natural sounds. The study noted that natural sounds had a higher statistical effect on several okra and zucchini seeds for the primary condition and overexposure time (Creath and Schwartz 2004). A study by Jeong*et al.* identified a group of responsive genes in plants using a set of sound frequencies as sound processing. It showed proper regulation through mRNA expression analyses, and experiments conducted under light and dark showed that sound could represent a substitute for light as a gene regulator. The mRNA expression increased significantly with treatment at 0.125 and 0.250 kHz but decreased considerably at 0.050 kHz. Furthermore, they suggested that specific phonemic processing frequencies in genetically modified (GM) plants can be used to regulate gene expression

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(Jeong*et al*., 2008).

This study aims to evaluate the effects of different volume of sounds of the Holy Qur'an on two varieties of cucumber using RAPD markers to detect changes in their phenotypic characteristics, the stability of the genetic material, and mutations.

Materials and methods of work

1. **Obtaining the samples**: Two varieties were obtained from the crop of cucumber of the hybrid (Aseel and Gazer) type, of American origin, by the farmer's office accredited to Dabana, which is in the form of seeds for two varieties.
2. **Design and conduct the experiment:** Transactions were cultivated in special cork sections in the beginning and after ten days germination they were transferred from the cork to the soil by three replicates for each experimental unit and each repeater consists of ten plants and each class has two treatments and the control group and after that the transactions were cultivated for the two varieties in the greenhouse assigned to the College of Science - Tikrit University on 9/1/2018. As for the control samples of the two cultivars, they were planted in the garden. The house hears regular sounds in the same way that the transplants were planted. They are arranged according to the symbols in Table (1). Where the symbol AC1 and BC1 refer to the original and prolific cultivars that were considered a control sample for molecular studies, DNA isolation was performed after ten days of germination, while the symbol AC2 and BC2 indicates the plants that heard regular sounds and that were done. Its cultivation in the home garden is considered a control sample for phenotypic and anatomical studies. The symbol AM1, BM1 refers to plants treated with a loud voice estimated at (200 HZ), while the symbol AM2, BM2 indicates plants treated with the voice with a low voice of approximately (100 HZ).

Table1. The treatment of the two varieties, the codes used, and the duration of exposure to the sound of Qur'an

Variety-Gazer				**Variety- Aseel**				**Name**
High volume	**Low volume**	**Normal sample**	**Control**	**High volume**	**Low volume**	**Normal sample**	**control**	**Treatment**
BM2	BM1	BC2	BC1	AM2	AM1	AC2	AC1	code
200 HZ	100 HZ	0HZ	0HZ	200 HZ	100 HZ	0HZ	0HZ	Sound frequency

sound. The control was planted in an environment outside the greenhouse, and was exposed to normal sounds. All plants of both cultivars heard and or exposed to the recitation of the complete Holy Qur'an with a high pitch (200 Hz) and a low pitch (100 Hz) for two months

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over five days per week at five hours per day from a registered device for sound. Meanwhile, a barrier was placed in front of the sound device to reduce low-treated plants' sound.

4. Molecular study: Samples AC1 and BC1 were collected ten days after germination. The rest of the other samples were collected after the plants have attained maturity and fruit-bearing after completing the exposure period. About 4-5 young leaves were taken from the growing top and placed in special bags marked and transferred to Laboratory directly to perform DNA isolation.

5. DNA extraction:DNA isolation was performed from young leaves using CTABaccording to the method described by (Weigand*et al*., 1993; Huang *et al*., 2013).

6. Measuring DNA concentration and purity: The process of measuring the DNA concentration was performed to estimate its purity using a NanoDrop device. The machine was first calibrated with samples dissolving solution before measurement. The test sample was softened in the dissolving solution to obtain a concentration of 50 ng/µL and kept frozen until use. A drop of the extracted genomic DNA was introduced into the machine and the reading was taken.

7. Deportation on agarose gel (Gel electrophoresis): Solutions, necessary materials, gel, and sample loading were prepared in the electrical relay process according to the method described by (Sambrook*et al*., 1989; Al Sukmani, 2017).

8. Conducting RAPD-PCR markers: The RAPD interactions were performed based on the method described by Williams et al. on the parameters and two control samples of the option plant using (12) primers (Williams *et al*., 1990), and the interactions were performed as shown in Table (2).

Table 2. Primeres used in the RAPD-PCR study

Primer name	sequence ˉ3 ˉ5	Primer name	sequence ˉ3 ˉ5
P-1	**GATGACCGCC**	**P-7**	**GTGTGCCCCA**
P-2	**ACTGGGACTC**	**P-8**	**GTCGCCGTCA**
P-3	**GACAGGAGGT**	**P-9**	**GTTGCGATCC**
P-4	**GGAGGGTGTT**	**P-10**	**AACGGTGACC**
P-5	**CCTTGACGCA**	**P-11**	**CAGCACCCAC**
P-6	**GGTCCCTGAC**	**P-12**	**GGAGTACTGG**

The DNA concentration was adjusted in all the samples studied by dilutINGwith a TE solution to obtain the concentration of approximately 50 ng /mL required to conduct RAPD

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markersfor each sample. The Master Reaction mixture was prepared by mixing the reaction components in a 2 mL sterile Eppendroffe tube. The mixture was spin in a Microfuge for 3-5 seconds to complete the mixing of the reaction components. The entire experiment was carried out in a sterile Hood, with gloves wornand tubes immersed in ice. The details are shown in 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 was performed as follows: The first denaturation was done for 1 cycle at 95 ºC for 5 min, then 45 cycles (denaturation at 93 ºC for 45 sec; annealing 36 ºC for 45 sec; and then extension at 72 ºC for 1.5 min) and final extension of 1 cycle at 72 ºC for 10 min. After the PCR amplification program, 4 µL of PCR products were separated using gel electrophoresis at a concentration of 1.5% with DNA markerafter the gel stained with ethidium bromide for 60 min. The products were visualized under a UV- transilluminator.

Diagnosis of mutations: The genetic variation in the DNA obtained from RAPD-PCR markers can be adopted to identify mutations in treatments compared with the control sample, and this is done by convertingthe bands which appeared in the gel electrophoresis to description table by putting (1) when there is a unique band and (0) at the absence of the band, that is, the band which appears in the treatment and control varieties.

Results and discussion

1. Morphological study results

The results of the studied phenotypes showed that portions of the crops and all of the Aseel variety were fully developed by day 31st November and sold within a period not exceeding a week. The control samples bloom on day 7th October, while a section of the plants exposed to the Qur'an on the 18th November, while a part of the control plants bore fruit on 25th October. We noted from the results in Table (4) that the highest number of leaves and nodes was in the BM1 plants exposed to the low sound of the Qur'an. In contrast, the fewest number of leaves and nodes were observed in the BM2 group exposed to the loud sound of the Qur'an. The number of fruits was highest in the AM2 group and lowest in the control

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group(AC2). The leaf width characteristic wasmost extensive in the control plant for the Gazer variety (BC2), and the lowest was in the treatment (BM2) group. Leaf length was highest in the treatment (AM2) group in the Aseel variety and the lowest in the treatment BM2 for the Gazer variety. With respect to height, plants attained the highest length in the treatment group (AM2) Aseel variety but the lowest in the control group(AC2) of the same variety.Of the six studied phenotypic results, it was observed that there is an apparent effect of the treatment of the Holy Qur'an on those traits. Most of the impact was positive, shown by the increase in the yield in line with some portions in the Qur'an. Our findings also revealed that 40% of the plants in two cultivars exposed to the loud volume sound of the Qur'an died compared to the control cultivars that had no deaths. On examinations of these plants by agricultural engineers, it was observed that there were no defects in the method of cultivation and no associated plant diseases. We, therefore, inferred deaths in these cultivars may be due to their intolerance to the sound from the Qur'an.

2. The results of the RAPD indicators

The results of the RAPD-PCR analysis to determine the RAPD markers in the experimental samples are shown in table 5. In all, 12 primers showed different patterns of bands. A total of 65 loci were identified on the sample gene, of which 7 were monomorphic loci and 58 polymorphic loci in all the samples. The primer P-5 was distinguished by the highest number of producing loci (9), and the primer P-6 was the least productive of the loci (3). The total numbers of bands produced from those loci were 269, of which 56 were the main band, and213 were polymorphic bands. The primer P-3 produced 46 bands, while primer P-11 produced 9 bands with a general variation of 90%. Most of the transactions, as shown in Tables (6), were distinguished by distinct bands (Unique band, Absent band). The total of distinctive mutantbands resulting from the primers produced in this study was (90) specific bands, of which (60) were unique and (30) Absent bands, BM2 transaction had the highest percentage of unique bands (14), and AM2 transaction had (11) bands. The absent bands of the BM1 transaction were 12. In contrast, the Transaction AM2 showed 9 absent bands, and those bands are distinctive. The distinctive bands are characteristics of these transactions which indicate the effect of the scientific miracle of the Holy Qur'an and the volume of sound on the genetic material (the DNA). It is of note that these bands were seen only in the samples exposed to sound. The induction of mutations in specific locations is responsible for identifying the primerand the emergence of the unique band. The band's appearance is consistent with many previous researchers' findings (Mba, 2013; Bradshaw, 2016; Guo*et al*., 2017). The primers varied in the resulting band's sizes. Their sizes ranged between (100-2750bp). The lowest molecular size (100bp) was in the P-11 primer, and the highest molecular size (2750bp) was in the P-3 primer.

A literature search showed that this is the first report on the effects of the volume of sound produced by reciting the Holy Qur'an on cucumber plant during the growth period. These effects were investigated at both the phenotypic and molecular level

Our study showed that the sound volume produced from reciting the Qur'an affect the genome of the cucumber and hence the genetic stability. The RAPD-PCR markers have demonstrated high efficiency in diagnosing these mutations in the cucumber plant. A small

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number of primers produced amounted to 12,which corresponds to the previous findings (AL-Assie, 2014; Horejsi and Staub, 1999). Transactions showed a difference in the number and quality of mutant bands with models that hear the Qur'an compared to the control samples and also to the volume of sound, which is significant on the two studied variety. This is also consistent with the findings of Hassanien (Hassanien, 2014).The Aseel variety (AM2)

Table 4. The phenotypic characteristics of the treated samples compared to the control group

Plant variety	Treatment	leaf number	Nod number	fruits number	leaf width cm	leaf length cm	Plant height cm
Aseel	AC2	25	18	18	9.5	11.5	27
	AM1	30	25	24	9.2	9.9	28.8
	AM2	35	30	45	9.9	11.85	100.6
Gazes	BC2	24	19	30	10	10.6	52
	BM1	40	40	34	9.4	11.4	62.6
	BM2	17	12	21	6	8	40.6

exposed to loud sound of the Qur'an exhibited several unique mutant bands (11), and the lowest number of bands was (2) in a few samples. Exposure of the samples to consecutively low and high sound produced (9) bands. This proves that listening to the Qur'an in a loud voice increases the occurrence of mutations. However, in the second variety (Gazer) bands, BM2 treatment, which hears the Qur'an loudly, exhibited 14 unique bands, and the lowest number of the representative sample reached 3 bands. As for absent bands, the BM1 transaction that hears the Qur'an in a low voice showed 12 bands. Samples AC2 and BC2 exposed to normal sounds exhibited little genetic stability changes, and the occurrence of mutations was insignificant. This may be due to the cucumber plant's sensitivity to sounds and everyday noise. This study demonstrates that exposure of the cucumber plant to a different volume of sound from the recitation of the Qur'an has a significant influence on the growth, yield and genetic stability of the plant. It also revealed that the volume of sound is directly proportional to the number of mutant bands. Plants are just like humans, and they interact with the sounds they are exposed to, sensitive to heat, cold, and light. They are also affected by noise like humans, and sound vibrations have been shown to affect plant growth (Ankur, 2016).

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Table (5): the number and type of loci, the number of bands producedand their type, andthe number of distinct bands and their type

S/N	PrimerNumber	Loci number	Monomorphic loci	Polymorphic loci number	Bands number	Monomorphic bands number	Polymorphic band number	Unique bands	Absent bands	variation ratio %
1	P-1	8	1	7	30	8	22	9	3	85
2	P-2	6	-	6	19	-	19	5	2	100
3	P-3	7	1	6	46	8	38	2	5	85
4	P-4	7	1	6	30	8	22	7	3	85
5	P-5	9	3	6	44	24	20	3	3	75
6	P-6	3	-	3	17	-	17	4	1	100
7	P-7	5	-	5	10	-	10	3	4	100
8	P-8	6	-	6	16	-	16	4	2	100
9	P-9	4	-	4	18	-	18	5	2	10
10	P-10	5	1	4	19	8	12	5	2	80
11	P-11	6	-	6	9	-	9	7	-	100
12	P-12	6	-	6	11	-	11	6	3	100
summation		65	7	58	269	56	213	60	30	90%

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Table (6): The distinct mutations treated in the two variety compared with the control sample

Primer name	Molecular weight	Gazer variety									Aseel variety						
		AC1		AC2		AM1		AM2			BC1	BC2		BM1		BM2	
	Bp	Unique	Absent	Unique	Absent	Unique	Absent	Unique	Absent	Unique	Absent	Unique	Absent	Unique	Absent	Unique	Absent
P-1	200-1600	-	-	1	-	2	-	2	-	2	-	-	-	1	1	1	1
P-2	500-2000	1	-	-	-	-	1	1	-	-	1	-	-	-	1	2	-
P-3	400-2000	-	-	-	-	-	-	1	3	-	-	-	-	1	2	-	-
P-4	250-1500	2	-	-	-	1	1	1	-	-	1	1	-	-	1	2	-
P-5	250-2750	-	-	-	-	1	1	-	-	-	-	-	-	1	-	1	1
P-6	1150-1500	-	-	-	-	-	1	-	1	-	-	-	-	-	2	1	-
P-7	400-1400	-	-	-	-	-	1	-	1	-	-	-	-	-	2	1	2
P-8	350-1500	1	-	-	-	-	1	-	1	1	-	-	-	-	-	2	-
P-9	400-1600	-	-	1	-	3	-	3	1	-	-	-	-	-	3	-	-
P-10	300-1500	-	-	-	-	1	-	-	1	1	1	2	-	-	-	-	-
P-11	100-1100	-	-	-	-	-	-	2	-	2	-	-	-	1	-	2	-
P-12	300-1500	1	-	-	1	-	1	1	1	1	-	1	-	-	-	2	-
summation	91	5	0	2	1	9	6	11	9	7	3	4	0	4	12	14	4

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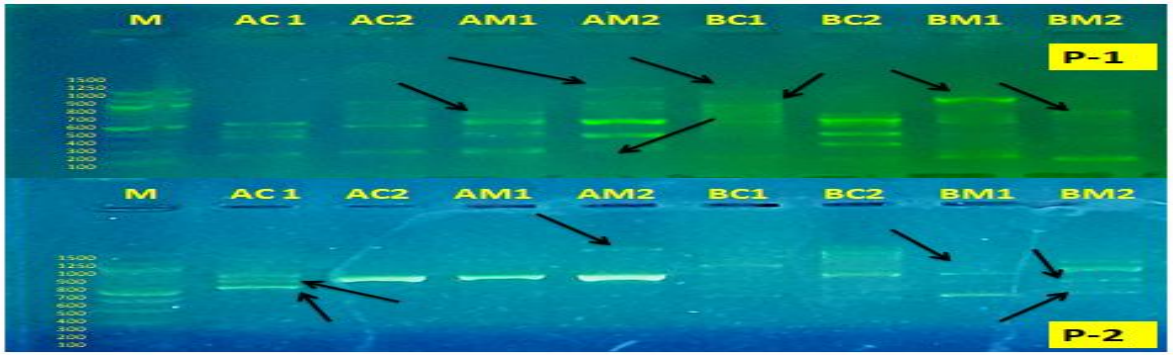
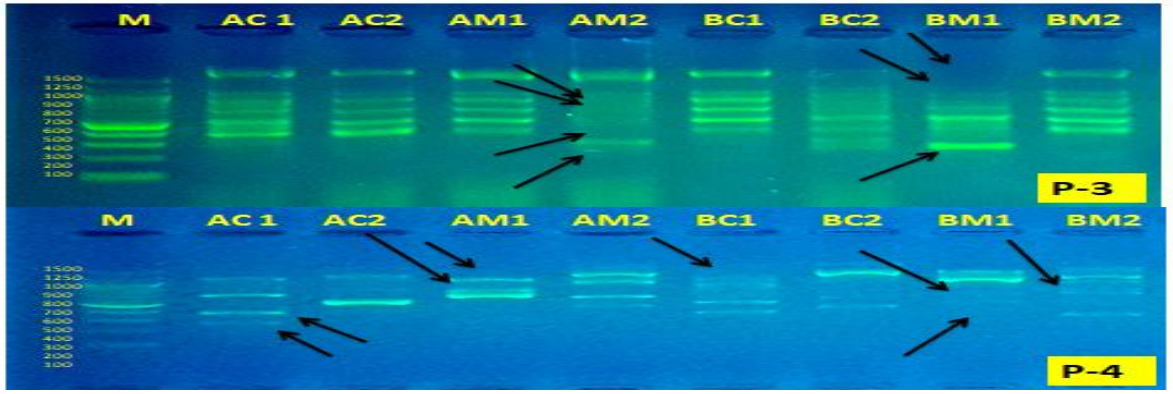


Picture (1) represents the Primer outcomes of P1,P2 with M marker



Picture (2) represents the Primer outcomes of P3,P4 with M marker

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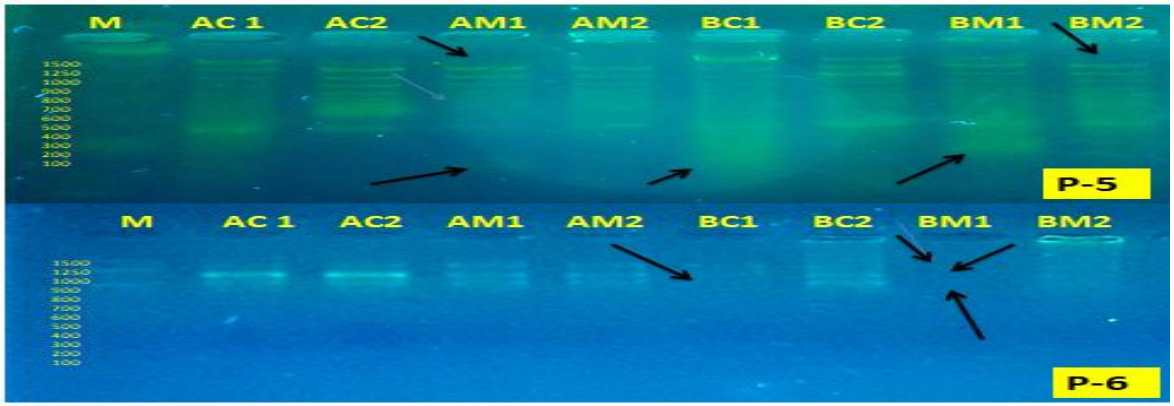
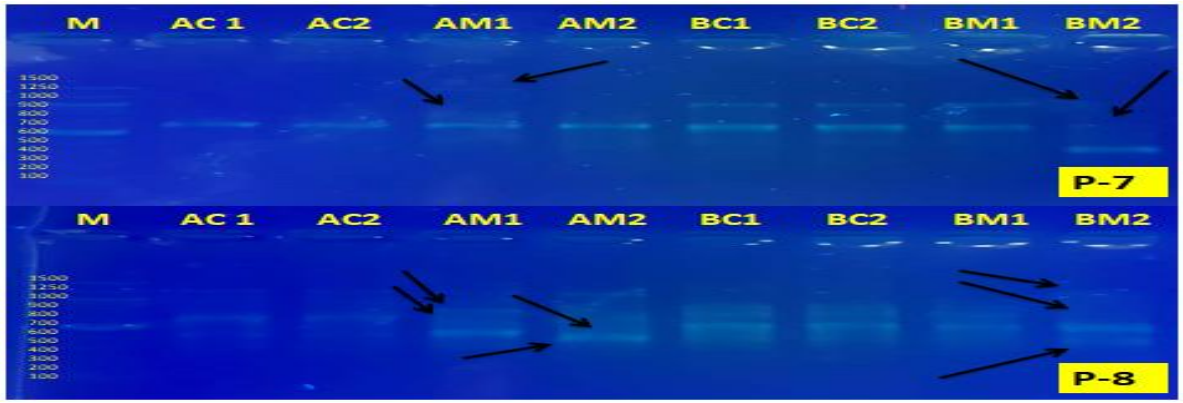


Picture (3) represents the Primer outcomes of P5,P6 with M marker



Picture (4) represents the Primer outcomes of P7,P8 with M marker

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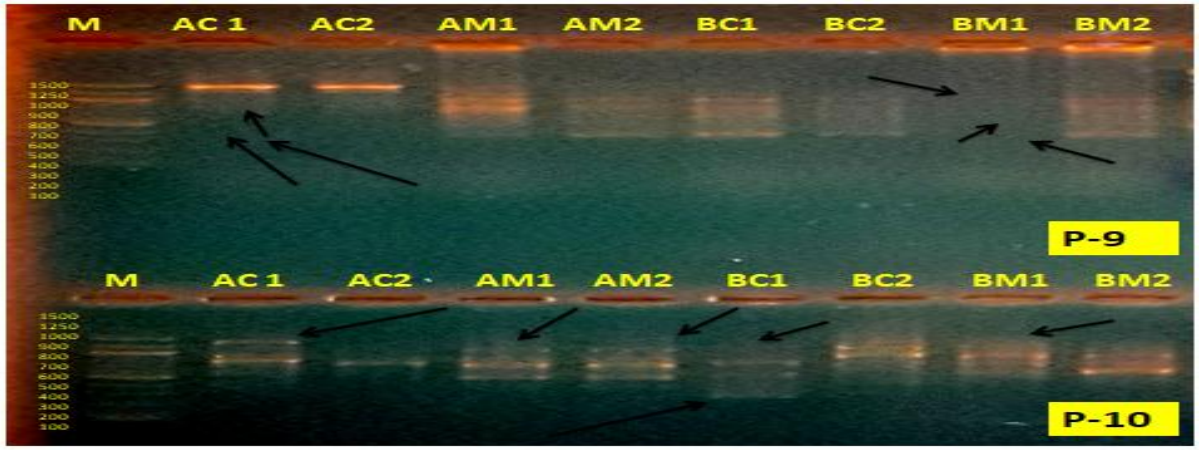
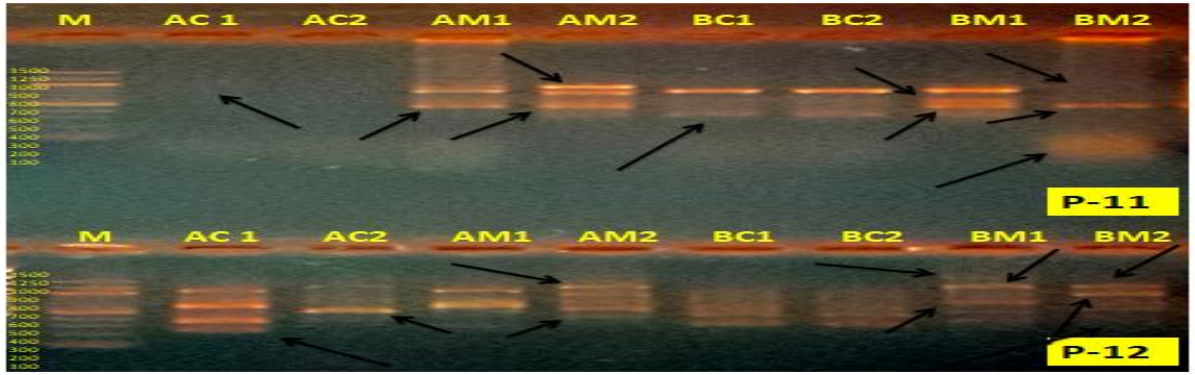


Picture (5) represents the Primer outcomes of P9,P10 with M marker



Picture (6) represents the Primer outcomes of P11,P12 with M marker

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There is an excellent agreement between the studied markers, especially phenotypic and molecular, in the pure variety from the previous results. The AM1 treatment was not significantly distinguished in the six studied phenotypes compared to the control sample and in molecular markers. It did not show a significant number of mutant bands except 9 unique bands and 6 absent bands. The second treatment, AM2 of an authentic variety, obtained the highest number of three phenotypic characteristics: the number of fruits, leaf length, and plant height compared to molecular markers where that treatment got 11 unique bands and 9 absentee bands. As for the second cultivar, it is prolific. The BM1 treatment in the phenotypic characteristics obtained the highest number of leaves and nodes compared to other transactions. In return, the highest number of absent mutant bands reached 12. In contrast, the second treatment for the prolific BM2 class, exposed to loud Quran sound, got the lowest number of leaves, nodes, width and length of leaves;however, it had the highest count Packages mutant total of 14 unique bands. These results show that the Qur'an exert significant and positive traits in a plant. In a study by Nakhavali and Seyedi, it was reported that an increase in sound frequencies above the usual limit of hearing in the human ear affects the transformation of sound waves and their translation in the brain negatively, and this could lead to depression and boredom, which applies to plants because they sense loud sounds (Nakhavali&Seyedi, 2013).

Conclusions

There is a significant influence of the sound volume of the Holy Qur'an's on the phenotypic markers on the genome of cucumber. The also revealed the adverse effects of high sound volume on the cucumber plant's phenotypic traits. The genome of the option wassignificantly affected by the increase in the pitch. Through the above results, it is clear that plants feel and speak, and their condition is affected by other organisms. Positive influences increase the plant's efficiency, production, vitality, and persistence in living and vice versa. Meanwhile, RAPD markers are efficientin detecting the resulting mutations in the genome of the option.

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