

ROLE OF COLCHICIN AND MANNITOL IN INDUCING GENETIC VARIATIONS AND DROUGHT TOLERANCE OF BARLEY VARIETIES IN VITRO

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ABSTRACT

This study was aimed to inducing genetic variations for drought tolerance in vitro for callus three varieties Ebaa265, Ebaa99 and Bouhoth244 of barley (*Hordeum vulgare* L.) using plant tissue culture technique. An experiment was conducted in plant tissue culture laboratory at the College of Agricultural Engineering Sciences, University of Baghdad during the years 2022-2023. The experiment included the use of colchicin at concentrations of (0,2,5,5 and 7.5) mg.L⁻¹ and for a soaking periods of (0,4,8 and 12) hours, and mannitol at concentrations of (0,2,4 and 6) g.L⁻¹ using MS media. The results showed that there were significant differences in the average dry weight of callus before and after colchicin treatment at a concentration of 7.5 mg.L⁻¹ for a soaking period of 12 hours and with mannitol 6 g.L⁻¹ and the comparison treatment. The dry weight of callus before colchicin treatment reached 40.30 mg in the variety Ebaa265, was significant different of the average dry weight of callus after colchicin treatment reached 92.65 mg within the variety Bouhoth244. The PCR-RAPD with five primers (OP-A06,OP-A07,OP-A08,OP-B01 and OP-B02) examination revealed differences in the number of resulting bands and their molecular weights in colchicin treatments and the comparison treatment, in light of the results. It could be concluded that colchicin has the ability to cause genetic variations and increase the tolerance of vegetative growth to drought stress resulting from treatment with the compound mannitol in Barley.

Keywords: plant tissue culture, callus, (PCR-RAPD).

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INTRODUCTION

The barley (*Hordeum vulgare* L.) crop was grown for various purposes, including using its grains in the manufacture of bread after mixing it with 5% wheat flour (Al-Khazraji ET AL, 2022). It was also used in the preparation of some food recipes, such as making soups, and in the manufacture of alcoholic beverages, in addition to using barley water for medicinal purposes because it contains high levels of dietary fiber and selenium (Jasmina & Marko, 2022). Plant tissue culture technology has become one of the most important techniques that resort to plant breeders. Especially in developing varieties that are tolerant to biotic and abiotic stresses, such as drought stress, and the possibility of improving the performance of

available varieties as one of the solutions to the problems that the world is witnessing, especially in the field of food security (Al-Jubori & Al-Amery, 2022). Therefore, the biological aspect has an important role in coexisting with the problem of drought. By improving the performance of available varieties using biological techniques such as in vitro cultivation technology as well as genetic engineering technology (Baday, 2018; Taha, 2020). Colchicin is known as an active substance that was extracted by the French chemists Pelletier and Caventon (1820) in pure form from the flowers of the autumn colchicum (*Colchicum autumnale* L.), which belongs to the *Liliaceae* family (antoshkumar et al, 2023). It is noteworthy that colchicin is a natural amino compound and is described as a

tricyclic alkaloid with a molecular weight of 7399.43 and is crystalline with a yellow color and a bitter taste (Ryan et al, 2020). Colchicin was used at a concentration of 0.05% in agricultural production to multiply chromosomes in plant cells for the purpose of producing industrial mutations characterized by abundant production and improving the characteristics, whether flowers or fruits of some economic medicinal plants, as this substance works to prevent the formation of spindle threads necessary for the process of chromosome division during the meiotic cycle, which leads to. Because the chromosomes remain in the center of the cell and then the genetic material is duplicated (Wee & Wei-Seng, 2019). Studies conducted by Pickett-Heaps (1966) confirm that colchicin has the ability to change cell shape, in addition to a noticeable change in chromosome behavior during the asexual division stage (Raufe et al, 2020). Azizan (Azizan et al, 2021) showed that when using concentrations of colchicin (0, 0.5, 1, 1.5, 2 and 2.5)% and for a soaking period of 48 hours, the results revealed that there were significant differences in the morphological characteristics of the stevia plants treated with concentrations of colchicin compared to the comparison treatment, as it achieved. The 2% concentration of colchicin had the highest average of plant height by an increase of 36%, an increase in leaf length by 43%, and an increase in leaf thickness by 5%. He confirmed that the plant characteristics had improved by increasing the concentrations of colchicin in stevia. Creating drought conditions through chemicals such as mannitol, Polyethylene glycol (PEG), and Sodium chloride (NaCl) in plant tissue culture technology can generate a wide range of genetic variations for different plant species, which can be introduced into breeding and selection programs for desired mutations and agricultural traits, such as tolerance to drought and salinity, or resistance to diseases and insects, and isolated in a short time. (Akila & Jayeni, 2023). (Taha, 2020) found that when using concentrations of mannitol (0.5, 10 and 15) mg.L⁻¹, the comparison treatment was superior to the rest of the mannitol (15, 20 and 25) atmospheric pressure on strains of barley

and wheat at the germination stage, and they indicated a noticeable decline in the amount of water absorbed by the seeds with the increase in the intensity of the applied stress, we showed that the barley strains were superior to wheat in terms of their tolerance to drought, in view of the overall indicators and characteristics studied, especially at the water stress levels of 20 and 25 atmospheres of pressure. They confirmed that the compound mannitol could be relied upon as a factor causing water stress in the germination medium, to screen the different genotypes of Barley wheat to tolerance average drought. Polymerase Chain Reaction (Random Amplified Polymorphic DNA) (PCR-RAPD) in which short random primers are used that can amplify several sites in the DNA molecule. It has been possible to develop several primers that are used for different purposes as a result of studies within the field of molecular biology. Usually, the length of the primer is about 10 bases, and nanogram amounts of Genomic DNA has low fusion temperatures using the denaturation reaction, which can be separated and stained with ethidium bromide stain. Therefore, species that are genetically close to each other show a similar distribution of bands, and species that are genetically distant from each other show a different distribution of bands (Al-Khazraji ET AL, 2022). (Al-Khazraji ET AL, 2022) indicated when evaluating the genetic diversity of six barley varieties (Samir, Al-Buraq, Amal, Shah, Al-Hadhar, IPA99) using the PCR-RAPD test with 10 random primers, the test revealed the presence of genetic variation amounting to 40%, and the analysis showed. The genetic distance of the varieties: The largest genetic distance between the two varieties Shah and Al-Buraq was 0.185, while the smallest genetic distance between the two varieties Hadar and Al-Amal amounted to 0.027. The examination using the PCR-RAPD technique is highly efficient in diagnosis and detecting the genetic connection between varieties, and it will also serve as an indicator for plant breeders. This study aims to evaluate the response of three barley varieties to drought tolerance by determining a concentration of Colchicin that stimulates

genetic variations and increases the tolerance of barley vegetative growths to drought stress.

MATERIALS AND METHODS

Three varieties of barley, Ebaa265, Ebaa99, and Bouhouth244, obtained from the General Company for Seed Testing and Certification, were used in this study. The seeds were used and soaked in colchicine at concentrations of (0, 2.5, 5 and 7.5) mg.L⁻¹ for (0, 4, 8 and 12) hours (Ni Made & Made, 2019). The seeds were then sterilized with 3% sodium hypochlorite (NaOCl) for 15 minutes (Baday, S. J. S. 2020), and the sterilized seeds were washed with sterile distilled water three times to remove traces of the sterile material in the stratified airflow table (5). In this study, ready-made (Murashige and Skoog) MS medium weighing 4.9 g.L⁻¹ was used in all stages of cultivation, prepared with (0, 1.5, 3 and 4.5) mg.L⁻¹ (Baday, S. J. S. 2020) of (2,4-Dichlorophenoxyacetic acid) 2,4-D for the purpose of callus induction, concentration 3 mg.L⁻¹ gave the highest average of wet and dry weight of callus, so it was used in the callus induction experiment. The pH of the nutrient medium was adjusted to 5.8, and sterilized with an autoclave for 20 minutes at a temperature of 121°C. The pressure was 1.04 kg.cm⁻¹, and the crops were incubated in the dark at a temperature of 25 ± 2°C. PCR test was conducted to determine the effect of colchicin in stimulating genetic variations. I used four concentrations of the compound Mannitol, which were added to the food medium during preparation in the following quantities: (0, 2, 4 and 6) g.L⁻¹ (Saadaoui et al, 2023). DNA genetic material was isolated from fresh callus of colchicin treatments according to the method (Dellaporta *et al*, 1983). I use the Wizard Genomic DNA Purification Kit to extract DNA, one sample for each type and concentration. It was used five random primers obtained from the American company Promega. Each primer consists of ten random nucleotide bases (OP-A06, OP-A07, OP-A08, OP-B01 and OP-B02), and included a DNA reaction mix (PCRPreMix). For one sample, dATP, dGTP, dCTP, dTTP (400 µm), Tris-HCl (50 mm), KCl (30 mm), MgCl₂ (3 mm), and Taq DNA Polymerase (50 Units. ml⁻¹) prepared from the

American company Promega, to it, 2 µl of primer, 2 µl of sample DNA, and 16 µl of sterile distilled water were added. The bands were separated averaged using a 2% agarose gel at a voltage of 70 volts for two hours, and by comparing the duplicated bands of the studied samples resulting from the RAPD reaction with the standard DNA bands and estimating their molecular sizes, the products of the RAPD reactions for DNA were obtained, as were the parameters of the studied species and according to the type of primer used. All experiments were carried out using a completely randomized design (CRD), with factorial experiments, the results were analyzed using the Genestat statistical program, and the means were compared according to the least significant difference test (LSD) at the 5% probability level (Al-Sahuki & Wahib, 1990).

RESULTS AND DISCUSSION

The effect of colchicin concentrations and soaking duration on the average dry weight of callus: The results in Table (1) show that there are significant differences between the varieties, as the variety Bouhuth244 achieved the highest average dry weight of callus, amounting to 75.49 mg, while the two varieties Ebaa99 and Ebaa265 achieved a lower average dry weight of callus, amounting to 51.66 and 48.34 mg, respectively. This could be attributed to the difference in the varieties in their content hormonal and genetic (horticultural plants, 2018). As for the concentration of colchicin, the concentration was significantly higher than 7.5 mg.L⁻¹ and gave the highest average dry weight of callus, amounting to 64.08 mg. This could be attributed to the fact that this concentration is the best in stimulating some of the desired characteristics of vegetative growth, in addition to this, the ability of colchicin to multiply chromosomes in plant cells for the purpose of production of artificial mutations characterized by abundant production and improved qualities, and this was confirmed by (Zidane & Al-Shamarey, 2014), while the comparison treatment gave the lowest average dry weight of callus, which amounted to 53.49 mg. It was revealed note that the soaking period of 12 hours was significantly superior,

as it had the highest average dry weight of callus, which reached 63.33 mg, and the lowest average dry weight of callus at the soaking period of 0 hours was 52.52 mg. As for the interaction between the varieties and the concentration of colchicin, significant differences were recorded, as the variety Bouhoth244, at a concentration of 7.5 mg.L⁻¹ gave the highest average dry weight of callus, amounting to 80.50 mg, while the lowest average dry weight of callus was recorded in the variety Ebaa265 when treated as a comparison, amounting to 42.72 mg. As for the interaction, Among the varieties and duration of soaking, the variety Bouhoth244 excelled at a soaking period of 12 hours in giving the highest average dry weight of callus, amounting to 81.63 mg, and the variety Ebaa265, at a soaking period of 0 hours, gave the lowest average dry weight of callus, amounting to 40.50 mg. The interaction between the concentration of colchicin and the duration of soaking, the highest average dry weight of callus was recorded at the

concentration of 7.5 mg.L⁻¹ of colchicin and the soaking duration was 12 hours, amounting to 74.32 mg. The lowest average dry weight of callus was recorded in the comparison treatment of colchicin and at the soaking duration of 0 hours, it amounted to 51.67mg. From the data in Table (1), we shows that there are significant differences between the varieties in the concentrations of colchicin and the duration of soaking, as the variety Bouhoth244 excelled at the concentration of 7.5 mg.L⁻¹ of colchicin and the duration of soaking was 12 hours, and gave the highest average dry weight of the callus, amounting to 92.65 mg. This could be due to the ability of colchicin to the chromosomes were doubled and thus the growth characteristics, such as fresh and dry weights, were doubled. This is consistent with the findings of (Azizan *et al.*,2021), while the Ebaa265 variety and the comparison treatment, at a soaking period of 0 hours the lowest average dry weight of callus recorded reached 40.30 mg.

Table 1. The effect of colchicin concentration (mg.L⁻¹) and soaking duration (hour) on the average dry weight (mg) of callus of barley varieties after six weeks of planting

Varieties	Colchicin concentration mg.L ⁻¹	Soaking time (h)				Varieties average× Colchicin concentration
		0	4	8	12	
Ebaa265	0	40.30	42.85	43.39	44.33	42.72
	2.5	40.35	47.31	48.60	51.75	47.00
	5	40.50	48.85	53.15	54.70	49.30
	7.5	40.85	50.25	59.35	62.85	53.33
Ebaa99	0	45.45	46.00	47.85	48.85	47.04
	2.5	46.15	49.70	50.80	53.30	49.99
	5	46.60	50.00	53.90	56.50	51.75
Bouhoth244	7.5	47.30	51.65	58.60	67.45	56.25
	0	70.00	70.45	70.70	70.85	70.56
	2.5	70.25	70.55	71.75	75.65	71.99
	5	70.90	71.25	71.85	80.35	73.36
	7.5	71.25	73.45	84.65	92.65	80.50
	LSD5%		0.56**			0.28**
	Varieties		Varieties×soaking time			Varieties concentration
	Ebaa265	40.50	47.32	51.12	54.41	48.34
	Ebaa99	46.38	49.70	53.29	57.28	51.66
	Bouhoth244	70.16	72.93	77.24	81.63	75.49
	LSD5%		0.09**			0.14**
	Colchicin concentration		Colchicin concentration× soaking time			Colchicin average concentration
	0	51.67	53.65	53.98	54.68	53.49
	2.5	52.05	56.19	57.05	60.23	56.38
	5	53.33	56.63	57.97	61.52	57.36
	7.5	53.80	60.00	68.20	74.32	64.08
	LSD5%		0.32**			0.16**
	Average soaking time	52.52	56.25	59.65	63.33	
	LSD5%		0.03**			

PCR-RAPR analysis

An appropriate amount of total DNA isolated and extracted from the tissues of barley varieties for the comparison treatment was obtained and the concentrations were (2.5, 5, 7.5) mg.L⁻¹ of colchicin and soaking for 12 hours. Five random primers were used, all of which succeeded in joining DNA sequences isolated from (0, 2.5, 5 and 7.5) mg.L⁻¹ of colchicin and was able to detect genetic variation between all treatments and varieties under study, Ebaa265, Ebaa99, and Bouhoth244, and the concentrations were (0, 2.5, 5 and 7.5) mg.L⁻¹ of colchicin and a soaking period of 12 hours because this period gave the highest values for the dry weight of the induced callus compared to the rest of the

treatments. Accordingly, the soaking period was chosen as 12 hours in conducting the PCR-RAPD examination and other periods were excluded.

Primer OP-A06

Table (2) and Figure (1) indicates that primer OP-A06 revealed the appearance of bands with weights of (2500), (1850), (1500), (800), and (700) bp in the comparison treatment and for all varieties, and their disappearance in all treatments from colchicin and for all concentrations, as colchicin affects DNA through its effect on the sequence of nitrogenous bases, as it works to form pyrimidine dimers through the formation of covalent bonds between cytosine and thymine bases (Ni Made & Made, 2019).

Table 2. Primer OP-A06 bands results for barley varieties treated with colchicin mg.L⁻¹ for 12 hours

Molecular weight bp	Colchicin mg.L ⁻¹											
	Bouhoth244				Ebaa99				Ebaa265			
	0	2.5	5	7.5	0	2.5	5	7.5	0	2.5	5	7.5
	13	14	15	16	17	18	19	20	21	22	23	24
2500	0	0	0	0	1	0	0	0	1	0	0	0
1850	1	0	0	0	1	0	0	0	1	0	0	0
1500	0	0	0	0	1	0	0	0	1	0	0	0
800	1	0	0	0	1	0	0	0	1	0	0	0
700	1	0	0	0	1	0	0	0	1	0	0	0

0: The band is absent 1: The band is present 1*: The band is bright 1**: The band is very bright

Bouhoth244 Ebaa99 Ebaa265

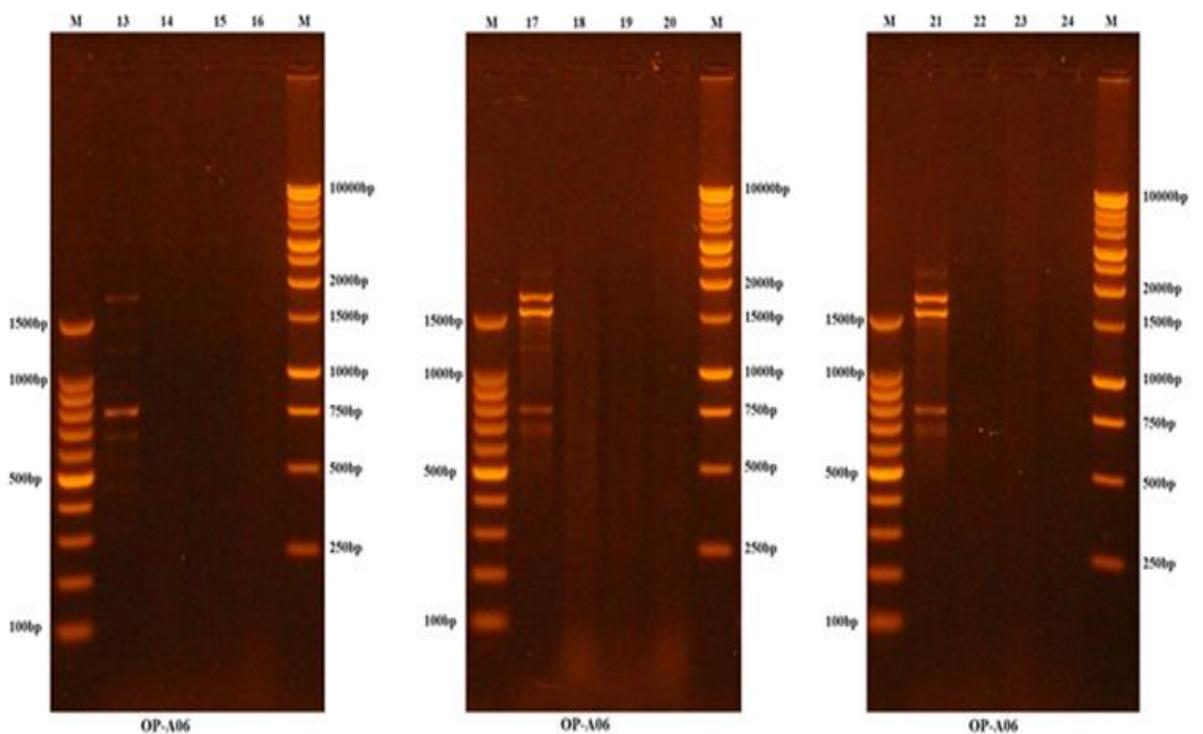


Figure 1. Results of PCR-RAPD analysis using primer OP-A06

Primer OP-A07

Table (3) and Figure (2) shows that the primer OP- A07 revealed the appearance of a band with a weight of (2000) bp at concentrations of 2.5 and 5 mg. L⁻¹ of colchicin in the variety Bohouth244, and its disappearance in all treatments and for the other two varieties, as well as the appearance of a band with a weight of (1600). bp at a concentration of 5 mg.L⁻¹ of colchicin in the variety Bouhoth244 and its disappearance in all treatments. For the other two varieties, a band with a weight of (1400) bp also appears. bp at concentrations of 2.5, 5 and 7.5 mg.L⁻¹ of colchicin in the variety Bouhoth244 and at a concentration of 5 mg.L⁻¹ of colchicin in the variety Ebaa99 at concentrations of 2.5 and 5 mg.L⁻¹ of the colchicin in the variety Ebaa265 and its disappearance in all other treatments. Two

bands with a weight of (600) and (500) bp appeared in the control treatment in all varieties and disappeared in all colchicin treatments. The reason for obtaining more than one band when using specialized primers is attributed to the possibility of an increase in the concentration of the DNA or the polymerizing enzyme, an increase in P, or an increase in 2+ in the concentration of magnesium ions MgP, the concentration of the initiator or the possibility of the difference in the bonding temperature of the initiator, as increasing the bonding temperature leads to the loss of many sites, and also lowering that temperature to less than the required limit may lead to the appearance of many bands that do not represent the reality of the sites. On the gene, but represent unspecialized reaction products (Khalifa *et al*, 2015).

Table 3. Primer OP-A07 bands results for barley varieties treated with colchicin mg.L⁻¹ for 12 hours

Molecular weight bp	Colchicin mg.L ⁻¹											
	Bouhoth244				Ebaa99				Ebaa265			
	0	2.5	5	7.5	0	2.5	5	7.5	0	2.5	5	7.5
	13	14	15	16	17	18	19	20	21	22	23	24
2000	0	1	1	0	0	0	0	0	0	0	0	0
1600	0	0	1	0	0	0	0	0	0	0	0	0
1400	0	1	1	1	0	0	1	0	0	1	1	0
1100	0	0	1	0	0	0	0	0	0	0	0	0
800	0	0	1	0	1	0	0	0	1	0	0	0
650	0	0	1	0	0	0	0	0	0	0	0	0
600	1	0	0	0	1	0	0	0	1	0	0	0
500	1	0	0	0	1	0	0	0	1	0	0	0
350	0	0	0	1	0	0	1	0	0	0	1	0

0: The band is absent 1: The band is present 1*: The band is bright 1***:The band is very bright

Bouhoth244 Ebaa99 Ebaa265

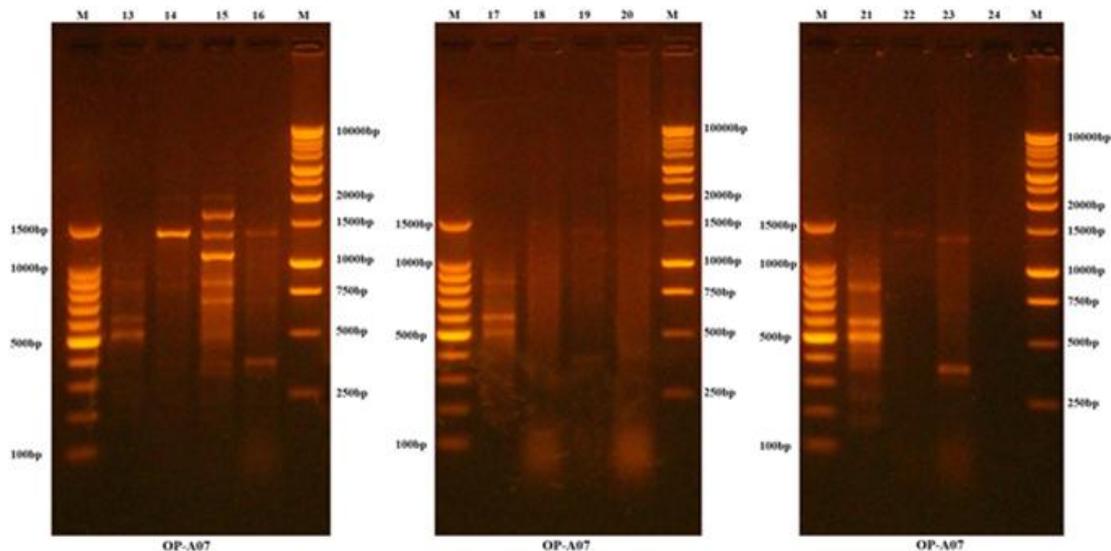


Figure 2. Results of PCR-RAPD analysis using primer OP-A07

Primer OP-A08

Table (4) and Figure (3) shows that the primer OP- A08 revealed the appearance of two bands with a weight of (1400) and (1300) bp in the comparison treatment in the variety Ebaa265, and their disappearance in all treatments and for the other two varieties, as well as the appearance of bright bands with a weight of

(1000) and (750) and (700) bp when treated with the comparison in the variety Bouhoth244 and Ebaa265 and they disappeared in all treatments and the variety Ebaa99, as well as the appearance of two fluorescent bands with a weight of (600) and (500) bp when treated with the comparison in all varieties and their disappearance in all treatments of colchicin.

Table 4. Primer OP-A08 bands results for barley varieties treated with colchicin mg.L⁻¹ for 12 hours

Molecular weight bp	Colchicin mg.L ⁻¹											
	Bouhoth244				Ebaa99				Ebaa265			
	0	2.5	5	7.5	0	2.5	5	7.5	0	2.5	5	7.5
	13	14	15	16	17	18	19	20	21	22	23	24
1400	0	0	0	0	0	0	0	0	1	0	0	0
1300	0	0	0	0	0	0	0	0	1	0	0	0
1000	1	0	0	0	0	0	0	0	*1	0	0	0
750	1	0	0	0	0	0	0	0	1	0	0	0
700	1	0	0	0	0	0	0	0	1	0	0	0
600	1	0	**1	0	1	0	0	0	1	0	0	0
500	1	1	0	0	0	0	0	0	1	0	0	0
200	0	1	0	0	0	0	0	1	0	0	0	1

0: The band is absent 1:The band is present 1*: The band is bright 1**:The band is very bright

Bouhoth 244 Ebaa99 Ebaa265

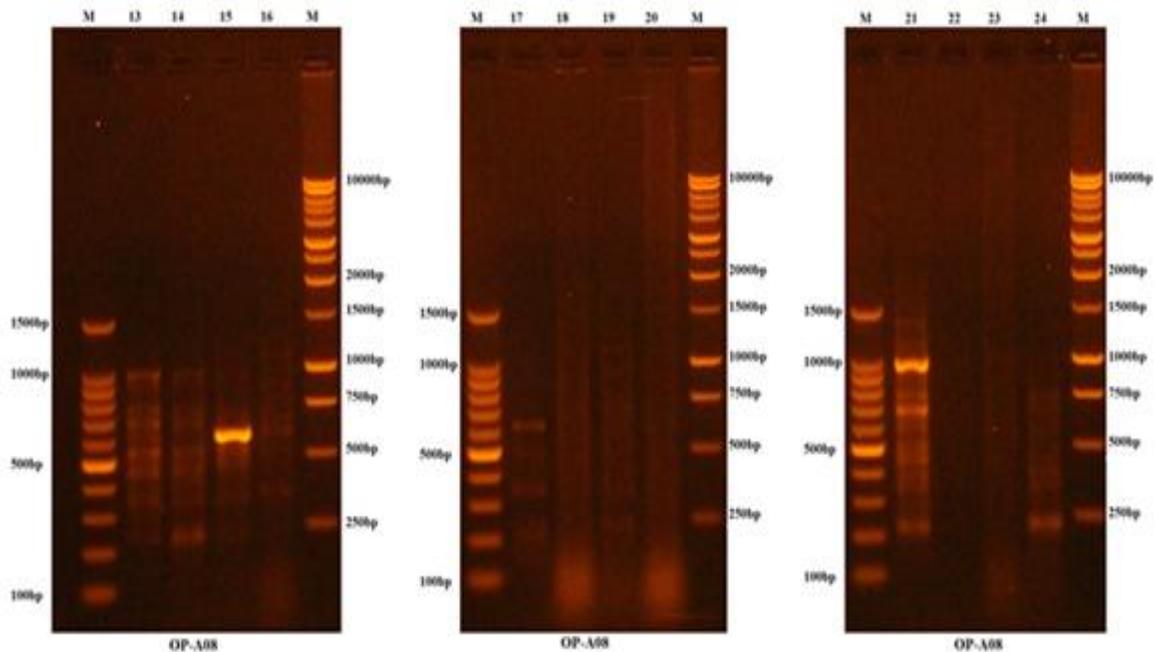


Figure 3. Results of PCR-RAPD analysis using primer OP-A08

Primer OP-B01

Table (5) and Figure (4) shows that primer OP-B01 revealed the appearance of bands weighing (800), (550), and (400) bp in the

comparison treatment and for all varieties, and their disappearance in all colchicin treatments and for all varieties.

Table 5. Primer OP-B01 bands results for barley varieties treated with colchicin mg.L⁻¹ for 12 hours

Molecular weight bp	Colchicin mg.L ⁻¹											
	Bouhoth244				Ebaa99				Ebaa245			
	0	2.5	5	7.5	0	2.5	5	7.5	0	2.5	5	7.5
	13	14	15	16	17	18	19	20	21	22	23	24
800	1	0	0	0	1	0	0	0	1	0	0	0
550	1	0	0	0	1	0	0	0	1	0	0	0
400	1	0	0	0	1	0	0	0	1	0	0	0

0: The band is absent 1: The band is present 1*: The band is bright 1** :The band is very bright

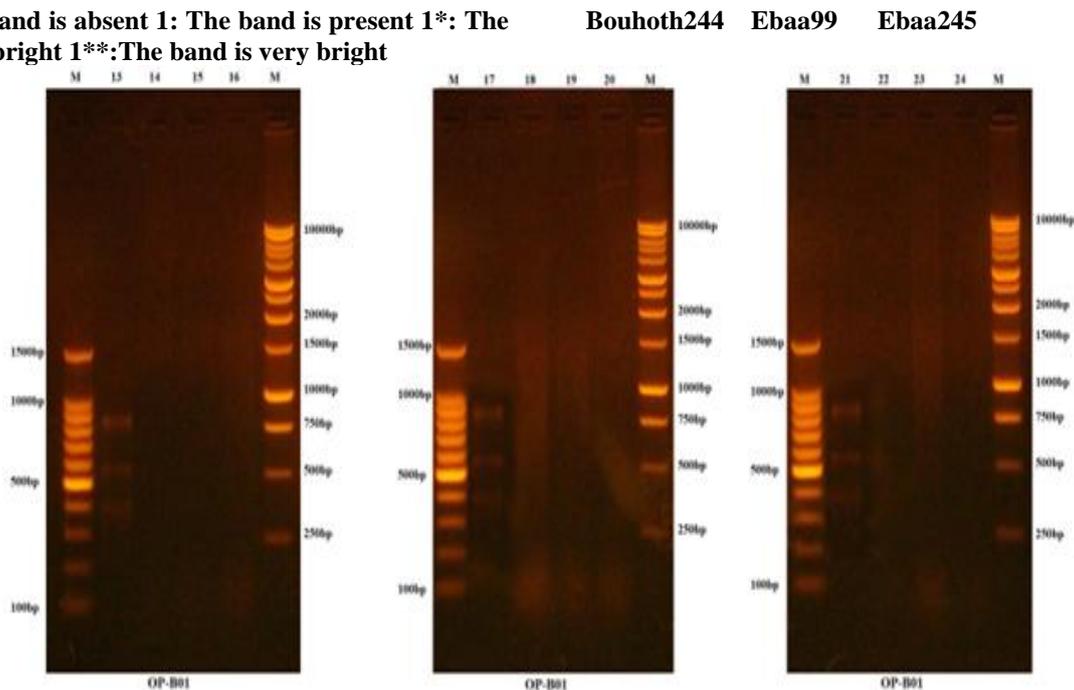


Figure 4. Results of PCR-RAPD analysis using primer OP-B01

Primer OP-B02

Table (6) and Figure (5) indicates that the primer OP-B02 revealed the appearance of a band with a weight of (1500) bp in the comparison treatment in the two varieties Ebaa99 and Ebaa265 and its disappearance in all treatments and the variety Bouhoth 244, as well as the appearance of two bands with a weight of (1400) and (1000) bp. At a concentration of 5 mg.L⁻¹ of colchicin in the variety Bouhoth244, it disappeared in all treatments and the other two variety s, as well

as the appearance of a band with a weight of (750) bp in the comparison treatment in the Ebaa99 and Ebaa265, and it disappeared in all treatments of colchicin and the variety Bouhoth244. Likewise, the appearance of a band with a weight of (200) bp at concentrations of 5 and 7.5 mg.L⁻¹ of colchicin in the two varieties Ebaa99 and Ebaa265 and its disappearance in all treatments of colchicin and in the two varieties Bouhoth244 and Ebaa265 and its disappearance in all treatments and the variety Ebaa99.

Table 6. Primer OP-B02 bands results for barley varieties treated with colchicin mg.L⁻¹ for 12 hours

Molecular weight bp	Colchicin mg.L ⁻¹											
	Bouhoth244				Ebaa99				Ebaa265			
	0	2.5	5	7.5	0	2.5	5	7.5	0	2.5	5	7.5
	13	14	15	16	17	18	19	20	21	22	23	24
1500	0	0	0	0	1	0	0	0	1	0	0	0
1400	0	0	1	0	0	0	0	0	0	0	0	0
1000	0	0	**1	0	0	0	0	0	1	0	0	0
750	0	0	0	0	1	0	0	0	**1	0	0	0
650	0	0	1	0	0	0	0	0	0	0	0	0
600	1	0	0	0	0	0	0	0	0	0	0	0
200	0	0	1	1	0	0	0	0	0	0	0	1

0: The band is absent 1: The band is present 1*: The band is bright 1** :The band is very bright

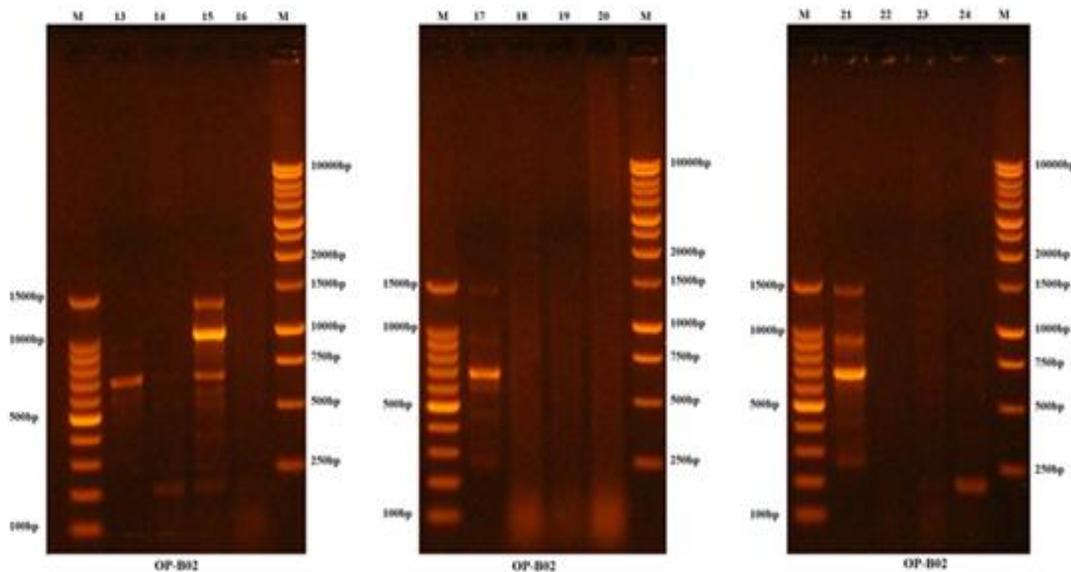


Figure 5. Results of PCR-RAPD analysis using primer OP-B02

The effect of mannitol concentrations on the average dry weight of callus resulting from soaking with colchicin at a concentration of 7.5 mg.L⁻¹ for 12 hours

The results in Table (7) show that there are significant differences between the barley varieties, as the variety Bouhoth244 was significantly superior in the average dry weight of callus, as it gave an average of 69.64 mg, compared to the two varieties Ebaa99 and Ebaa265, which gave a lower average dry weight of 56.60 and 50.40 mg, respectively. The variation between varieties is due to genetic differences among them and to the difference in the content of internal hormones and their role in various growth processes (Al-

taei *et al.*,2019). As for the effect of mannitol concentrations on the average dry weight of callus, the results in Table (7) indicate that there are significant differences between the concentrations of mannitol, as it was observed that there was a gradual decrease in the average dry weight of callus with an increase in the concentration of mannitol, as the highest average dry weight of callus was achieved in the control treatment. of mannitol amounted to 74.67 mg. The reason for the superiority of the control treatment in the average dry weight of callus may be due to the effect of mannitol. These results were confirmed by what the researchers reached (Khierallah &Jawad, 2017; Najm &Hamza,2023), while the average

dry weight of callus decreased to 37.00 mg when increased the concentration of mannitol reached 6 g.L⁻¹. The decrease in the average dry weight of callus at high concentrations of mannitol may be due to the fact that high concentrations of mannitol may lead to inhibition of cell growth and may stop them from dividing and expanding (Al-taei &Hamza,2020; Al-taei &Hamza, 2023). These results were confirmed. When he found it (Akila &Jayeni,2023; Taha,2020). It is noted

from the same table that the interaction between varieties and mannitol concentrations indicates the presence of significant differences, as the lowest average dry weight of callus was recorded in the Ebaa265 variety, amounting to 32.00 mg at a concentration of 6 g. L⁻¹ of mannitol, while the highest average dry weight of callus was achieved in the Bouhoth244 variety, amounting to 87.80 mg in treating the comparison .

Table 7. Effect of Mannitol concentration (g.L⁻¹) on the average dry weight (mg) of callus resulting from soaking seeds of barley varieties with Colchicin 7.5 (mg.L⁻¹) for (12) hours after four weeks of planting

Mannitol concentration g.L ⁻¹	Varieties			Average concentration of Mannitol
	Ebaa265	Ebaa99	Bouhoth244	
(without treatment)	48.20	51.00	61.20	53.47
0	64.40	71.80	87.80	74.67
2	56.40	65.80	81.80	68.00
4	51.00	57.80	75.00	61.27
6	32.00	36.60	42.40	37.00
LSD5%		0.47**		0.27**
Varieties average	50.40	56.60	69.64	
LSD5%		0.21**		

CONCLUSION

The colchicin have efficient ability to cause genetic variations in the varieties of barely used (Bouhoth244, Ebaa99 and Ebaa265) by increasing the tolerance of vegetative plants to drought stress resulting from treatment with mannitol.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

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دور الكولشسين والمانيتول في إستحثاث التغيرات الوراثية وتحمل الجفاف لأصناف من الشعير خارج الجسم الحي

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المستخلص

تهدف الدراسة إلى إستحثاث التغيرات الوراثية وتحمل الجفاف في كالس ثلاثة أصناف من الشعير: إباء 265 وإباء 99 وبحوث 244 بإستعمال تقانة زراعة الأنسجة النباتية، أجريت التجربة في مختبر زراعة الأنسجة النباتية في كلية علوم الهندسة الزراعية - جامعة بغداد خلال السنتين 2022-2023. إشملت التجربة على إستعمال الكولشسين بالتراكيز (0، 2.5، 5، 7.5) ملغم. لتر⁻¹ ومدّة النقع (0، 4، 8، 12) ساعة، والمانيتول بإستعمال بالتراكيز (0، 2، 4، 6) غم. لتر⁻¹ وإستعمال الوسط الغذائي MS. بينت النتائج وجود فروق معنوية في معدل الوزن الجاف للكالس قبل وبعد المعاملة بالكولشسين عند التركيز 7.5 ملغم. لتر⁻¹ لمدة النقع 12 ساعة والمانيتول 6 غم. لتر⁻¹ ومعاملة المقارنة. بلغ معدل الوزن الجاف للكالس قبل المعاملة بالكولشسين 40.30 ملغم في الصنف إباء 265، والذي إختلف معنوياً عن معدل الوزن الجاف للكالس بعد المعاملة بالكولشسين بلغ 92.65 ملغم في الصنف بحوث 244. كشف فحص PCR-RAPD مع خمس بادئات (OP-A06، OP-A07، OP-A08، OP-B01 و OP-B02) وجود إختلافات في عدد الحزم الناتجة وأوزانها الجزيئية في معاملات الكولشسين ومعاملة المقارنة، وفي ضوء النتائج يمكن الإستنتاج أن الكولشسين له القدرة على إحداث التغيرات الوراثية وزيادة تحمل النموات الخضرية لإجهاد الجفاف الناتج عن المعاملة بمركب المانيتول في الشعير.

الكلمات المفتاحية: زراعة الأنسجة النباتية، الكالس، (PCR-RAPD).

*جزء من أطروحة دكتوراه للباحث الاول.