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## THE EFFECT OF HARMEL SEED EXTRACT ON THE VEGETATIVE GROWTH CHARACTERISTICS AND DROUGHT TOLERANCE OF SUNFLOWER *IN VITRO*

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## ABSTRACT

The world has recently become focused on finding alternatives to plant growth regulators, particularly those used in plant tissue culture, as they are the cornerstone of establishing tissue cultures and are involved in all stages of agriculture. Plant extracts are one of the most important factors that encourage growth, as they contain many active compounds that can replace hormones and increase plant resistance to drought. This study aimed to use harmala extract as an alternative to artificial hormones and to induce genetic variations that would increase the ability of plants to tolerance stress. In this study, sunflower embryos were grown in media known as Murashige and Skoog (MS) culture media that containing (0, 4, 6 and 8) ml L<sup>-1</sup> of alcoholic extract of *Peganum harmel* seeds, as compared to benzyl adenine (BA) treatments of (0, 1, 2, and 3) mg L<sup>-1</sup> and indole acetic acid (IAA) of 0.5 mg L<sup>-1</sup>. The plantlet that recorded the highest average number of vegetative shoots and dry weight were transferred to a culture medium containing mannitol compound from (0, 5, 10, and 15) g L<sup>-1</sup>. The results experimentally showed that the actual highest average number of vegetative shoots formed was 22.6 shoots plant<sup>-1</sup>, and the total amount of chlorophyll was 983.9 mg g<sup>-1</sup>, and the dry weight of vegetative shoots was 303.4 mg at 6 ml L<sup>-1</sup> of harmel seed extract and 2 mg L<sup>-1</sup> of BA in the Elf variety. The relative average growth was 66.2 mg day<sup>-1</sup>, while the plant growth average was 73.8 mg day<sup>-1</sup> and the stress tolerance index was 2.321 in the Elf variety and the 6 ml g<sup>-1</sup> of harmel seed extract and 10 g L<sup>-1</sup> of mannitol. The polymerase chain reaction-rapid amplification polymerase DNA (PCR-RAPD) revealed a difference in the number of bands and their molecular weights for the harmel extract treatments compared to the comparison treatment.

**Key words:** Peganum harmel, BA, stress, mannitol, PCR-RAPD, tissue culture

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## INTRODUCTION

Sunflower is the third most important oil crop in day life use and a member of the star family. It is characterized by its large, radial flowers that rotate with the sun, hence its name. It is cultivated as an ornamental plant, and its seeds are edible. Sunflower seeds contain chlorides, 35-55% oil, small amounts of fluorine, and vitamins A and B [1].

Plant growth regulators are expensive compounds. In Iraq, they are imported because they are not manufactured locally [2, 3, 4]. It is necessary to develop cheaper, locally available materials that improve vegetative growth of plants. Natural plant extracts that can improve plant growth are important [5, 6].

Genetic variations could be induced especially by plant tissue culture by using plant extracts, therefore, researchers paid higher attention to the study of these extracts [7]. The plant extracts could affect tissue-cultured plants [8] and determine whether they can replace plant growth regulators such as *Peganum harmel* seed extract [9], which contains many important glycosides and alkaloids, especially alkaloids. Alkaloids are defined as organic compounds present in plants that have at least one nitrogen atom in the hydrocarbon ring.

Most of them have a bitter taste, and they have an importance in the ionic balance in the plant instead of inorganic bases. Harmel seed extract also contains many alkaloid compounds like B-carbolines. Alkaloids include harmaline, harmine, quinazoline alkaloids, pegamine alkaloids, and quinazoline [10, 11, 12].

This also impacts photosynthesis and stomata function. This effect in cell water relationships, such as water potential, osmotic potential, and turgor potential, causes stomata to close, thus reducing the amount of carbon dioxide (CO<sub>2</sub>) entering the cells [13, 14, 15].

The cause of the drought problem has encouraged many researchers to understand the mechanisms that help plants adapt to drought conditions, such as selecting varieties with genetic traits for drought resistance [16, 17]. Drought-tolerant plants are characterized by their ability to grow with low soil water content, stable membranes, low photosynthesis, high levels of soluble sugars and proline [18, 19], accumulation of complex amino acids, higher concentration of chlorophyll molecules, and accumulation of enzymatic and non-enzymatic antioxidants [20, 21].

This was achieved by controlling the water irrigation, the period of irrigation, or by the using of organic compounds that stimulate water tension like mannitol and polyethylene glycol (PEG) [22, 23]. The solution concentration depends upon the principle of drawing water away from the plant because of the potential difference between the internal and external environments [24].



Drought caused by osmotic stress affects a wide range of problems in growth, water status, alterations in transporting systems and ions found in plants [25, 26, 27]. Osmotic pressure can be gradually raised by adding sugar alcohol to the culture media. Sugar has a significant effect on osmotic pressure [28, 29], the growth and emergence of plant organs in the artificial environment can stop due to the inability of the plant part to absorb water [30].

The aim of the work is to improve the vegetative growth characteristics and increase the plant tolerance to resist drought stress. This will be conducted by adding the extract of harmel seed as an alternative to chemicals represented by cytokinins that are responsible for increasing cell division like benzyl adenine (BA) and auxins responsible for cell elongation like indole acetic acid (IAA).

## **MATERIALS AND METHODS**

### **Varieties**

Mammoth and Elf Seeds were obtained from the Seed Inspection and Certification Department/ Agricultural Research Department/ Ministry of Agriculture [31]. Mammoth is the giant of the sunflower family It can reach over 3 meters in height and is characterized by its large, seed-filled head. This variety is often grown for its edible seed production and for adding dramatic appeal to large gardens. Elf is a dwarf variety, standing only 45 cm tall. Its compact size makes it ideal for smaller gardens, pots, or as a decorative border along walkways. Despite its small size, Elf produces dense heads of bright yellow petals, adding a touch of elegance and beauty without requiring a lot of space.

### **Preparation for extract of harmel**

The dried harmel seeds were ground, 100g of powder was weighed, and 1000 mL of methanol was added to it. It was left to soak for 2 hours to allow the active ingredients to be extracted. The mixture was filtered, and the filtrate was then collected and concentrated using rotary evaporator, where it was left until the volume reached 100 mL. The extract was filtered using Whatman No. 1 filter paper, the pH of nutrient medium was adjusted to be 5.8 before adding the extract and sterilizing the entire medium. The extract was sterilized by filtration through 0.22  $\mu\text{m}$  membrane filters after heat sterilization of the basic medium to preserve heat-sensitive compounds according to Baday and Hamza [32].

### **Preparation of seeds and media**

Seeds were sterilized using mercuric chloride at 1 g L<sup>-1</sup> for 10 minutes, seeds were sterilized by using mercuric chloride (HgCl<sub>2</sub>) 1 g L<sup>-1</sup> for 10 minutes according to Sawant et al. [33]. Murashige and Skoog [34] media was used as a culture were sterilized and autoclaved at 121°C for 15 minutes [35].



## Culture and acclimation

Sunflower embryos were planted in media containing the methanolic extract of harmel seeds at (0, 4, 6 and 8) ml L<sup>-1</sup>, and regulators BA of plant growth at (0, 1, 2 and 3) mg L<sup>-1</sup> and IAA at 0.5 mg L<sup>-1</sup> [36].

Plantlets were transferred this was by using 6 ml L<sup>-1</sup> of harmel extract, and 2 mg L<sup>-1</sup> of BA to nutrient media containing mannitol at 0, 5, 10 and 15 g L<sup>-1</sup> [37], the cultures were incubated at 25°C and using light with intensity equal to 1000 lux for sixteen hours of lighting and eight hours darkness [38].

Data such as the number of branches, dry weights, and other traits were recorded four weeks after planting. The next stages, such as rooting and acclimatization, were then carried out for field examination. The young plants were removed from the tubes, the roots were gently washed to remove any residual growth medium, and the plants were planted in vermiculite and soil and placed in small pots according to Baday [39].

## Measurement of dry weight and number of shoots

Measurements were recorded after 28 days of cultivation on a nutrient medium containing concentrations of harmel extract and boric acid. Five samples were collected for each concentration and variety, based on the number of buds present in each plant with the total number of buds per sample, then divided by the number of samples measured according to Dere et al. [40].

The cultures were extracted from the bottles in which they were grown and placed on filter paper. The remaining culture medium was removed using a surgical blade, and five samples were collected for each concentration and variety. The samples were dried in an oven at a constant temperature of 60–80°C for 24–48 hours until they reached a constant weight.

## Measurement of PRGR and PGR

The physiological characteristics were measured as the average relative growth according to Farshadfar et al. [41] using plant relative growth rate (PRGR) equation as shown below:

$$(\text{PRGR}) = (W_2 - W_1) / W_1$$

Where W<sub>1</sub> and W<sub>2</sub> are the primal weight of plants before and after four 28 days, respectively, of cultivated embryos on MS media was accurately measured at 7, 14, 21 and 28 days after transferring plant to media and measuring index tolerant drought.



## PCR-RAPD

Three random primers obtained from the American company, Promega were used. Each primer consists of ten random nucleotide bases and includes a DNA reaction mix (PCRPreMix).

For each sample 2  $\mu$ l of primer, 2  $\mu$ l of sample DNA, and 16  $\mu$ l of sterile distilled water were added. Tris-HCl (50 mmol), KCl (30 mmol), MgCl<sub>2</sub> (3 mmol), and Taq DNA Polymerase (50 Units ml<sup>-1</sup>) were purchased from Promega, United States.

The bands were separated using a 2% agarose gel at 70 volts for two hours, by comparing the duplicated bands of the studied samples resulting from the RAPD reaction with the standard DNA bands, their molecular sizes were estimated.

The products of the RAPD reactions for DNA were obtained, according to the parameters of the studied species and according to the type of primer [42].

## Data analysis

The work was performed from a fully Complete Randomized Design (CRD). The sample size on which the analysis was conducted was 160 samples resulting from the use of two varieties of sunflower, 4 concentrations of harmel extract, 4 concentrations of BA, and 5 replications. Data analysis was performed using a factorial experiment design with a (CRD) and five replications for each treatment, concentration, and variety across all experiments. The averages were analyzed using Least Significant Difference (LSD) test at 0.05 probability level, as implemented in the Genestat 2007 program [43].

## RESULTS AND DISCUSSION

### Effect of harmel extract and BA concentrations on the number of vegetative shoots

Table 1 indicates a high difference among the two sunflower types. The Elf exhibited the highest average number of shoots up to 12.9 shoots plant<sup>-1</sup>. The Mammoth achieved a lower average number of shoots at 9.1 shoots plant<sup>-1</sup>. The extract of Harmel seed concentrations shows a high superiority of the 6 ml L<sup>-1</sup>, with the highest average number of shoots which was 15.1 shoots plant<sup>-1</sup>, while the control treatment exhibited a lower average number of shoots that reached 8.7 shoots plant<sup>-1</sup>. The 2 mg L<sup>-1</sup> of BA significantly exhibited the highest average number of shoots, 16.0 shoots plant<sup>-1</sup>, while control treatment showed a very low average number of shoots 9.0 shoots plant<sup>-1</sup>. The Elf at 6 ml L<sup>-1</sup> gave the highest average number of shoots up to 17.8 shoots plant<sup>-1</sup>. The superiority of harmel seed extract concentrations in increasing the number of shoots compared to the control treatment is attributed to the fact that this extract contains hormones, while the lowest average number of shoots, 6.4 shoots plant<sup>-1</sup>, was recorded when using Mammoth at 0 ml L<sup>-1</sup>. This result



attributed to the genetic and hormonal differences between varieties [44]. The Elf at 2 mg L<sup>-1</sup> attained a higher average number of shoots 18.0 shoots plant<sup>-1</sup>, while the Mammoth at 0 mg L<sup>-1</sup> of BA afford a lower average number of shoots 6.9 shoots plant<sup>-1</sup>, as shown in Figure 2. Regarding the interaction between the concentrations of harmel seed extract and BA concentrations, the highest average number of shoots was recorded as 6 ml L<sup>-1</sup> of harmel seed extract and 2 mg L<sup>-1</sup> of BA, amounting to 19.2 shoots plant<sup>-1</sup>, while the 0 of harmel seed extract and BA gave the lowest average number of shoots at 6.2 shoots plant<sup>-1</sup>. Table 1 shows a significant difference between the varieties, concentrations of harmel seed extract and concentrations of BA. The Elf was highest at 6 ml L<sup>-1</sup> of harmel seed extract and 2 mg L<sup>-1</sup> of BA, with the highest average number of shoots up to 22.6 shoots plant<sup>-1</sup>, while the Mammoth showed the lowest average number of shoots at 5.6 shoots plant<sup>-1</sup>, at 0 ml L<sup>-1</sup> of harmel seed extract and BA, as in Figure 1 and 2.

### **Effect of harmel extract and BA concentrations on dry weight**

Table 2 indicates significant differences between the two sunflower varieties. The Elf achieved the highest average shoot dry weight of 242.6 mg, while the Mammoth achieved a lower average branch dry weight of 160.1 mg. Harmel seed extract concentrations registered 6 ml L<sup>-1</sup>, yielding the highest shoots dry weight of 230.0 mg, while the control treatment recorded a lower average shoot dry weight of 180.2 mg. The 2 mg L<sup>-1</sup> concentration of BA was the highest, yielding the highest average shoot dry weight of 234.1 mg, while the treatment of control showed the lowest average shoot dry weight of 175.1 mg. The interaction between varieties and harmel seed extract concentrations was higher. The Elf at 6 ml L<sup>-1</sup> gave the highest average dry weight of shoots up to 266.8 mg. The Mammoth at 0 ml L<sup>-1</sup> gave the lowest average dry weight of shoots at 134.9 mg. For the interaction between varieties and concentrations of BA where the Elf at 2 mg L<sup>-1</sup> gave the highest average dry weight of shoots at 278.2 mg, while the Mammoth at 0 mg L<sup>-1</sup> concentration using BA afforded the lowest average dry weight of shoots at 134.3 mg. As regards the interaction among the concentrations of harmel seed extract and BA concentrations where the highest average dry weight of shoots recorded was 6 ml L<sup>-1</sup> of harmel extract and 2 mg L<sup>-1</sup> of BA at 294.2 mg, while the concentration of 0 of harmel extract and BA afforded the lowest average dry weight of shoots, amounting to 170.2 mg. The Elf gave a higher performance at concentration of 6 ml L<sup>-1</sup> of harmel extract and 2 mg L<sup>-1</sup> of BA, and afforded the highest average dry weight of shoots at 303.4 mg, while the Mammoth recorded 0 ml L<sup>-1</sup> of harmel seed extract and BA the lowest average dry weight of shoots at 127.2 mg.



### **Effect of mannitol in plantlets that achieved highest number of shoots, total chlorophyll and dry weight treated harmel extract and BA on plant relative growth rate**

Table 3 shows significant differences between the two grain varieties, where Elf exceeded at 6 ml L<sup>-1</sup> of harmel extract and was dominated with plant relative growth rate and gave 51.3 mg day<sup>-1</sup> compared to Mammoth, at 2 mg L<sup>-1</sup> of BA, which gave a lower plant relative growth rate of 29.3 mg day<sup>-1</sup>. The effect of mannitol on the relative plant growth rate is shown in Table 4 where the results reveal that there were significant differences caused by mannitol where the highest plant relative growth rate appeared at 50.6 mg day<sup>-1</sup> was achieved at 10 g L<sup>-1</sup> of mannitol, while plant relative growth rate lowered to 35.2 mg day<sup>-1</sup> when the mannitol increased to 15 g L<sup>-1</sup>. This decreasing of plant relative growth rate may be drought produced to the addition of mannitol, that causes lowering in photosynthesis necessary for carbohydrates and proteins building [45]. Table 4 shows the interaction between the varieties and mannitol where the lowest plant relative growth rate was achieved in Mammoth at 2 mg L<sup>-1</sup> of BA, was 29.3 mg day<sup>-1</sup> 6 ml L<sup>-1</sup> of harmel extract was 66.2 mg day<sup>-1</sup> at 10 g L<sup>-1</sup> of mannitol, which may be attributed to the nature of these cells to the stress tolerance, and plasticity that DNA has in the cells of plant to form genetic structures that are more resistant to stress conditions [37]. The decrease in the plant relative growth rate at high of Mannitol may be attributed to a decrease in cell division, a decrease in cytoplasmic volume, loss of cell turgor, and nutritional imbalance due to a decrease in water and nutrient absorption, an increase in electrical potential, and a decrease in cellular water content with increasing stress. Perhaps the increase in stress causes physical damage to the carbon metabolism mechanism because of exposure to continuous stress, or perhaps it is attributed to the fact that the cytoplasmic content of the plant cell becomes denser, and its volume decreases when the cell is deficient in water [46, 47].

### **Effect of mannitol in plantlets that achieved highest number of shoots, total chlorophyll and dry weight treated harmel extract and BA on index tolerant drought**

Table 4 results show considerable differences between sunflower varieties, as the Elf variety significantly outperformed the plant in the average stress tolerance index, giving an average of 1.306 compared to the Mammoth, which gave a lower average stress tolerance index of 0.822. The results in the table record the highest average stress tolerance index for callus of 10 g L<sup>-1</sup> of mannitol, amounting to 1.797. These results are confirmed by what was reached by earlier work [48-52], while the average stress tolerance index decreased to 0.529 when the mannitol concentration increased to 15 g L<sup>-1</sup>. The table indicated that the interaction between the varieties and mannitol concentrations the lowest average stress tolerance index was recorded at Mammoth, reaching 0.453 of 15 g L<sup>-1</sup> of mannitol, while the highest average stress



tolerance index was achieved in the Elf, reaching 2.321 of 10 g L<sup>-1</sup> of mannitol, as in Figures 3 and 4.



**Figure 1: The Elf is treated with harmel seed extract**



**Figure 2: Elf is treated with BA**



**Figure 3: Tissue culture**



**Figure 4: Tissue culture**

### **The primer OPA-5**

The results in Table 5 and Figure 5 indicate the number of bands and their molecular weights resulting from the use of OPA-5 primer. The primer revealed a band with a molecular weight of 650 bp in the harmel treatments and it disappeared in the comparison treatment.

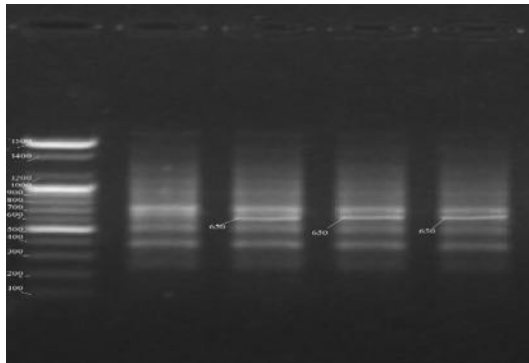
### **The Primer OPA-11**

The results from Table 6 and Figure 6 indicated OPA-11 primer revealed the number of bands resulting and their molecular weights for the treatments 4, 6, 8 ml L<sup>-1</sup> of harmel extract as well as the comparison treatment. The treatments produced bands whose molecular weights ranged between (250-1500) bp, and a band with a molecular weight of 500 bp was revealed in the harmel treatments and it had disappeared in the comparison treatment.

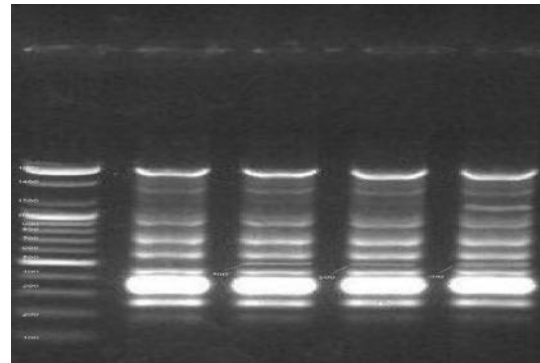
### **The Primer OPZ-11**

Table 7 and Figure 7 indicated that the OPZ-11 primer showed the number of resulting bands and their molecular weights for the treatments 4, 6, and 8 ml L<sup>-1</sup> of harmel extract and the control treatment. The treatments produced bands with

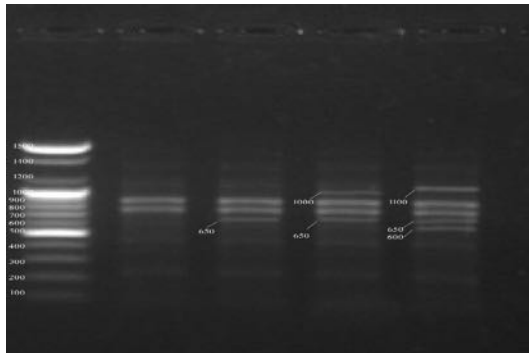
molecular weights ranging between (300-1100) bp. Two bands with molecular weights of 1100 and 600 bp were detected in the 8 ml L<sup>-1</sup> treatment of harmel, and they disappeared in the rest of the treatments. A band with a molecular weight of 1000 bp was detected in the 6 ml L<sup>-1</sup> treatment of harmel, and it disappeared in the rest of the treatments. A band with a molecular weight of 650 bp was detected in the harmel treatments, and it disappeared in the control treatment.



**Figure 5: Results of PCR-RAPD analysis using primer OPA-5**



**Figure 6: Results of PCR-RAPD analysis using primer OPA-11**



**Figure 7: Results of PCR-RAPD analysis using primer OPZ-11**

## CONCLUSION AND RECOMMENDATIONS FOR DEVELOPMENT

The harmel seed extract treatments were superior in the measurements of physiological characteristics such as the average number of shoots, the total amount of chlorophyll and the dry weight attributed to the harmel extract containing keloids. Which is the foundation for building cells, increasing growth and division and increasing the tolerance of varieties to drought stress because the extract containing antioxidants and reduce ROS. Conclude from the study that the harmel extract could

induce genetic variations that would increase tolerance to tissue-induced drought stress in sunflowers, which is one of the aims of sustainable development.

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**Table 1: Effect of concentrations of harmel extract and BA on the number of vegetative shoots plant<sup>-1</sup>**

Varieties	BA conc.	Harmel extract conc.				Varieties average × BA conc.
		0	4	6	8	
Mammoth	0	5.6	6.8	8.0	7.0	6.9
	1	6.8	8.4	9.6	8.6	8.4
	2	9.6	11.6	14.8	13.4	12.4
	3	7.6	9.6	13.2	11.8	10.6
Elf	0	6.8	8.8	15.6	12.4	10.9
	1	10.0	11.6	16.0	14.6	13.1
	2	12.5	15.6	22.6	21.2	18.0
	3	11.4	12.4	20	16.4	15.1
LSD0.05		15.3				11.2
Varieties		Harmel extract × varieties				Varieties average
Mammoth		6.4	8.1	11.2	10.5	9.1
Elf		9.3	11.1	17.8	13.4	12.9
LSD0.05		11.2				3.6
BA conc.		Harmel extract × BA				BA average
0		6.2	7.8	11.3	10.7	9.0
1		7.9	10	13.8	11.1	10.7
2		12.3	14.6	19.2	17.8	16
3		9.0	11.0	17.6	13.1	12.7
LSD0.05		12.3				7.5
Harmel average extract		8.7	10.5	15.1	13.0	
LSD0.05		7.5				

**Table 2: Effect of concentrations of harmel extract and BA on the dry weight mg**

Varieties	BA conc.	Harmel extract conc.				Average of varieties × BA conc.
		0	4	6	8	
Mammoth	0	127.2	127.8	141.4	140.6	134.3
	1	136.4	146.8	167.2	162.0	153.1
	2	138.8	176.4	285.0	178.6	194.7
	3	137.2	148.4	179.4	167.2	158.1
Elf	0	213.2	215.8	218.6	216.4	216.0
	1	219.4	219.6	266.6	225.2	232.7
	2	254.2	267.4	303.4	287.6	278.2
	3	230.0	242.4	278.6	276.2	256.8
LSD0.05		173.4				123.3
Varieties		<b>Harmel extract × varieties</b>				<b>Varieties average</b>
Mammoth		134.9	149.9	193.3	162.1	160.1
Elf		215.7	236.3	266.8	251.4	242.6
LSD0.05		123.3				82.6
BA conc.		<b>Harmel extract × BA</b>				<b>BA average</b>
0		170.2	171.8	180.0	178.5	175.1
1		177.9	183.2	216.9	193.6	192.9
2		187.0	221.9	294.2	233.1	234.1
3		181.1	195.4	229.0	221.7	206.8
LSD0.05		134.8				95.3
Harmel average extract		180.2	193.1	230.0	206.7	
LSD0.05		95.3				



**Table 3: Effect of Mannitol in plantlets that achieved highest number of shoots, total chlorophyll and dry weight treated harmel extract and BA on plant relative growth rate mg day<sup>-1</sup>**

Varieties Mannitol Conc.	1	2	3	4	Average
0	43.2	33.5	41.5	30.4	37.2
5	54.5	37.3	52.3	33.6	44.4
10	66.2	41.5	58.3	36.2	50.6
15	41.3	30.2	40.1	29.3	35.2
LSD0.05	37.8				15.5
Average	51.3	35.6	48.1	32.4	
LSD0.05	19.3				

1. Elf treated with 6 ml L<sup>-1</sup> of harmel extract
2. Elf treated with 2 mg L<sup>-1</sup> of BA
3. The Mammoth treated with 6 ml L<sup>-1</sup> of harmel extract
4. The Mammoth treated with 2 mg L<sup>-1</sup> of BA

**Table 4: Effect of mannitol in plantlets that achieved highest number of shoots, total chlorophyll and dry weight treated harmel extract and BA on index tolerant drought**

Varieties Mannitol Conc.	1	2	3	4	Average
5	0.931	0.822	0.911	0.801	0.866
10	2.321	1.542	2.112	1.212	1.797
15	0.665	0.467	0.532	0.453	0.529
LSD0.05	43.6				19.4
Average	1.306	0.944	1.185	0.822	
LSD0.05	21.6				

1. Elf treated with 6 ml L<sup>-1</sup> of harmel extract
2. Elf treated with 2 mg L<sup>-1</sup> of BA
3. The Mammoth treated with 6 ml L<sup>-1</sup> of harmel extract
4. The Mammoth treated with 2 mg L<sup>-1</sup> of BA

**Table 5: Number of resulting bands and estimated molecular weight (bp) using the OPA-5 primer for tissues of the Elf variety treated with concentrations of harmel extract**

Harmel extract concentrations ml L <sup>-1</sup>							
Cont.		4		6		8	
M.wt bp	Bands number	M.wt bp	Bands number	M.wt bp	Bands number	M.wt bp	Bands number
1400	1	1400	1	1400	1	1400	1
1300	1	1300	1	1300	1	1300	1
1200	1	1200	1	1200	1	1200	1
900	1	900	1	900	1	900	1
700	1	700	1	700	1	700	1
<b>650</b>	<b>0</b>	<b>650</b>	<b>0</b>	<b>650</b>	<b>1</b>	<b>650</b>	<b>1</b>
500	1	500	1	500	1	500	1
400	1	400	1	400	1	400	1
250	1	250	1	250	1	250	1

**Table 6: the number of bands resulting and the estimated molecular weight (bp) using the OPA-11 primer for tissues of the Elf variety treated with concentrations of harmel extract**

Harmel extract concentrations ml L <sup>-1</sup>							
Cont.		4		6		8	
M.wt bp	Bands number	M.wt bp	Bands number	M.wt bp	Bands number	M.wt bp	Bands number
1500	1	1500	1	1500	1	1500	1
1300	1	1300	1	1300	1	1300	1
900	1	900	1	900	1	900	1
700	1	700	1	700	1	700	1
600	1	600	1	600	1	600	1
<b>500</b>	<b>0</b>	<b>500</b>	<b>1</b>	<b>500</b>	<b>1</b>	<b>500</b>	<b>1</b>
450	1	450	1	450	1	450	1
400	1	400	1	400	1	400	1
300	1	300	1	300	1	300	1
250	1	250	1	250	1	250	1

**Table 7: number of resulting bands and estimated molecular weight (bp) using the OPZ-11 primer for tissues of the Elf variety treated with concentrations of harmel extract**

Harmel extract concentrations ml L <sup>-1</sup>							
Cont.		4		6		8	
M.wt bp	Bands number	M.wt bp	Bands number	M.wt bp	Bands number	M.wt bp	Bands number
1100	0	1100	1	1100	0	1100	1
1000	0	1000	0	1000	1	1000	0
850	1	850	1	850	1	850	1
700	1	700	1	700	1	700	1
650	0	650	1	650	1	650	1
600	0	600	0	600	0	600	1
550	1	550	1	550	1	550	1
400	1	400	1	400	1	400	1
350	1	350	1	350	1	350	1
300	1	300	1	300	1	300	1

## REFERENCES

1. **Puttha R, Karthikeyan V, Sayomphoo H, Jittimon W, Thanya P, Pao S, Kanokporn P and C Narin** Exploring the Potential of Sunflowers: Agronomy, Applications, and Opportunities within Bio-Circular-Green Economy *Horticulturae*. 2023; **9 (10)**: 1079.  
<https://doi.org/10.3390/horticulturae9101079>
2. **Nasralla AY, Khairallah HSM and SI Neema** The effect of plant growth regulators on stimulating the production of plant antioxidants from buckwheat plant callus. *Iraqi Journal of Agricultural Sciences*. 2015;**46(6)**: 922-933.
3. **Akila WY and HB Jayeni** Tissue Culture A Sustainable Approach to Explore Plant Stresses. *Life*, 2023;**13**: 780. <https://doi.org/10.3390/life13030780>
4. **Jha P, Ochatt S, Kumar J and V Wuschel** A master regulator in plant growth signaling. *Plant Cell Rep*. 2020; **39**:431-444.
5. **Mamedov NA, Pasdaran A and NZ Mamadalieva** Pharmacological of Syrian rue (*Peganum harmel* L. Zygophyllaceae). *Int. J. Second. Educ.*, 2018; **5**: 1-6.
6. **Baday SJS** *In Vitro* Genetic Variations of Two Rice Varieties Seeds Using (*Citrullus colocynthis*) Fruits Extract. *Plant Archives*. 2018;**18 (2)**: 2181-2186.
7. **Makhuvele R, Naidu K, Gbashi S, Thipe VC, Adebo, OA and PB Njobeh** The use of plant extracts and their phytochemicals for control of toxigenic fungi and mycotoxins. *Heliyon*. 2020; **6(10)**: 1-11.
8. **Giannenas P, Sidiropoulou E, Eleftherios B, Christaki E and P Florou-Paneri** Chapter 1 - The history of herbs, medicinal and aromatic plants, and their extracts: Past, current situation and future perspectives. *Aromatic Plants and Herbs in Animal Nutrition and Health*. 2020;1-18.
9. **Shahrajabian MH and W Sun** Wild Rue (*Peganum harmel*), an Extraordinary Source of Natural Products and Pharmacological Benefits. *Biol. Life Sci. Forum*. 2023; **26**: 2-5.
10. **Ingrisano R, Tosato E, Trost P, Gurrieri L and F Sparla** Proline, Cysteine and Branched-Chain Amino Acids in Abiotic Stress Response of Land Plants and Microalgae. *Plants*. 2023; **12**: 1-15.



11. **Hasanuzzaman M, Bhuyan MHMB, Zulfiqar F, Raza A Mohsin SM Mahmud JA Fujita M and V Fotopoulos** Reactive Oxygen Species and Antioxidant Defense in Plants under Abiotic Stress: Revisiting the Crucial Role of a Universal Defense Regulator. *Antioxidants*. 2020; **9(8)**: 1-52.
12. **Hasanuzzaman M, Raihan MRH, Masud AAC, Rahman K, Nowroz F, Rahman M Nahar K and M Fujita** Regulation of Reactive Oxygen Species and Antioxidant Defense in Plants under Salinity. *Int J Mol Sci.*, 2021; **22(17)**: 1-30.
13. **Lama DI and SI Neamah** Metabolic responses to *in vitro* in drought-tolerant in a (*Cucurbita pepo* L.) elicited by salicylic acid and zinc oxide nanoparticles. *Iraqi Journal of Agricultural Sciences*. 2023; **54(5)**: 1223-1233. <https://doi.org/10.36103/ijas.v54i5.1817>
14. **Saleem MH, Wang X, Parveen A Perveen S, Mehmood S Fiaz S, Ali S Hussain S Adnan M Iqbal N Alatawi A and S Ali** Alleviation of drought stress by root-applied thiourea is related to elevated photosynthetic pigments, osmoprotectants, antioxidant enzymes, and tubers yield and suppressed oxidative stress in potatoes cultivars. *Peer J*. 2022; **10**: 1-24.
15. **Yavas I, Jamal MA, Din KU, Ali S, Hussain S and M Farooq** Drought-Induced Changes in Leaf Morphology and Anatomy: Overview, Implications and Perspectives. *Pol. J. Environ. Stud.*, 2024; **33 (2)**: 1517 -1530.
16. **Shareef HJ and JM Al-Khayri** Salt and drought stress exhibits oxidative stress and modulated protein patterns in roots and leaves of date palm (*Phoenix dactylifera* L.). *Acta Agriculturae Slovenica*, 2021;**117(1)**: 1-10.
17. **Hussain S, Ulhassan Z, Brestic M, Zivcak M, Zhou W, Allakhverdiev SI, Yang X Safdar ME, Yang W and W Liu** Photosynthesis research under climate change. *Photosynthesis Research*. 2021;**150**: 5-19.
18. **Spormann S, Nadais P, Sousa F, Pinto M, Martins M, Sousa B, Fidalgo F and C Soares** Accumulation of Proline in Plants under Contaminated Soils—Are We on the Same Page? *Antioxidants*. 2023; **12**:1-26.
19. **Nour M M, Aljabi HR, AL-Huqail A A Horneburg B Mohammed AE and MO Alotaibi** Drought responses and adaptation in plants differing in life-form. *Frontiers in Ecology and Evolution*. 2024; **12**. <https://doi.org/10.3389/fevo.2024.1452427>



20. **Khatun M, Sarkar S, Era FM, Islam AKMM, Anwar MPF, Zhao W, Liu L, Shen Q, Yang J, Han X, Tian F and J Wu** Effects of Water Stress on Photosynthesis, Yield, and Water Use Efficiency in Winter Wheat. *Water*. 2020; **12**: 1-19.
21. **Hamza I A and H Ali** Effect of seed soaking with GA3 on emergence and seedling growth of corn under salt stress. *Iraqi Journal of Agricultural Sciences*. 2017; **48(3)**: 650-659.
22. **Qayyum A, Al Ayoubi S, Sher A, Bibi Y, Ahmad S, Shen Z and AJ Matthew** Improvement in drought tolerance in bread wheat is related to an improvement in osmolyte production, antioxidant enzyme activities, and gaseous exchange. *Saudi Journal of Biological Sciences*. 2021; **28(9)**: 5238-5249.
23. **Nimra Z, Nudrat AA, Kaneez F, Sibgha N, Muhammad SA, Saliha U, Muhammad A, Abdulaziz AA and M Sheikh** Drought-induced changes in plant-yield interlinked biochemistry of cauliflower (*Brassica oleracea* L. var. botrytis) by exogenously applied alpha-tocopherol. *Journal of King Saud University - Science*, 2024; **36(1)**.  
<https://doi.org/10.1016/j.jksus.2023.103028>
24. **Zhao W, Liu L, Shen Q, Yang J, Han X, Tian F and J Wu** Effects of Water Stress on Photosynthesis, Yield, and Water Use Efficiency in Winter Wheat. *Water*. 2020; **12**: 1-19.
25. **Kumari VV, Banerjee P, Verma VC, Sukumaran S, Chandran MAS, Gopinath KA, Venkatesh G, Yadav SK, Singh VK and NK Awasthi** Plant Nutrition: An Effective Way to Alleviate Abiotic Stress in Agricultural Crops. *Int J Mol Sci.*, 2022; **23(15)**: 1-30.
26. **Pepe M, Crescente MF and L Varone** Effect of Water Stress on Physiological and Morphological Leaf Traits: A Comparison among the Three Widely Spread Invasive Alien Species *Ailanthus Altissima*, *Phytolacca Americana*, and *Robinia Pseudoacacia*. *Plants*, 2022; **11**: 899.
27. **Chen M, Zhang W, Wu H, Guang C and W Mu** Mannitol: physiological functionalities, determination methods, biotechnological production, and applications. *Appl Microbiol Biotechnol*. 2020; **104(16)**: 6941-6951.



28. **Checa J and JM Aran** Reactive Oxygen Species: Drivers of Physiological and Pathological Processes. *J Inflamm Res.* 2020; **2(13)**: 1057-1073. <https://doi.org/10.2147/JIR.S275595>
29. **Khierallah H and H Jawad** Evaluation response of eight potato cultivars *in vitro* growth under salt stress conditions to *Iraqi Journal of Agricultural Sciences*, 2017; **48(6)**: 1612-1632. <https://doi.org/10.36103/ijas.v48i6%20B.22>
30. **Wang X and H Feng** Investigating the Role Played by Osmotic Pressure Difference in Osmotic Dehydration: Interactions between Apple Slices and Binary and Multi-Component Osmotic Systems. *Foods.* 2023; **12**: 1-28.
31. **Database of Agricultural Varieties and Hybrids.** National Committee for the Accreditation, Registration, Certification and Protection of Agricultural Varieties - Fourth Edition - Iraq. 2022. <https://zeraa.gov.iq/?page=13> Accessed March 2025.
32. **Baday SJS and IA Hamza** The Role of Harmel Seed Extract and Mannitol in Inducing Genetic Variations and Drought Tolerance of Barley Varieties *in Vitro.* *IOP Conference Series: Earth and Environmental Science*, 2024; **1371**, *Techniques of Agricultural Economic and Social Sciences.* <https://doi.org/10.1088/issn.1755-1315>
33. **Sawant GB, Sawardekar SV, Patil MY and SM Jadhav** Surface sterilization and callus induction potential of various indica rice (*Oryza sativa* L.) varieties. *Applied Biological Research.* 2023; **23(2)**: 147-156.
34. **Murashige T and F Skoog** A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant.* 1962; **15**: 473-797.
35. **Al-Jubori M T, AL-Dabbagh FMK and EW Alani** Optimizing media sterilization via chlorine dioxide and autoclaving of paulowni micropropagation. *Iraqi Journal of Agricultural Sciences.* 2023; **54(6)**: 1737-1745.
36. **Al-Jubori MT and L Al-Amery** Effect of cytokinins BA, Kin, auxin IAA and IBA on propagating (*Citrus aurantiifolia*) *in vitro.* *Int. J. Agricult. Stat. Sci.*, 2022; **18(1)**: 2033-2040.

37. **Saadaoui W, Neji T, Insaf M, Ourania P, Hanen F, Chadha A, Roua A, Riadh K and AP Spyridon** Effects of drought stress induced by D-Mannitol on the germination and early seedling growth traits, physiological parameters and phytochemicals content of Tunisian squash (*Cucurbita maxima* Duch.) landraces. *Front Plant Sci.* 2023; **31(14)**: 1215394.  
<https://doi.org/10.3389/fpls.2023.1215394>
38. **Ikram S, Javed F, Wahid A and R Ahmad** *In vitro* relationship between cadmium stress and thiourea in two barley genotypes. *Pak. J. Agri. Sci.*, 2014; **51(3)**: 673-678.
39. **Baday SJS** Sugarcane of rapid multiplication by callogenesis. 1st International Conference on Pure Science (ISCPS-2020). *Journal of Physics: Conference Series* 1660, 012005. IOP Publishing. 2020;  
<https://doi.org/10.1088/1742-6596/1660/1/012005>
40. **Dere S, Gunes T and R Sivaci** Spectrophotometric determination of chlorophyll- A, B and total carotenoid contents of some algae species using different solvents. *Tr. J. of Botany*, 1998;**22**: 13-17.
41. **Farshadfar E, Jamshidi B and M Chehri** Assessment of immature embryo culture to select for drought tolerance in bread wheat. *Int. J. Bio sci.*, 2014; **4(4)**: 194-203.
42. **Kantipudi NB, Thotten ES, Divakaran M, Muliya KR and PK Illathidath** Random amplified polymorphic DNA (RAPD) and derived techniques. *Molecular Plant Taxonomy.* 2021; 219–247. [https://doi.org/10.1007/978-1-0716-0997-2\\_13](https://doi.org/10.1007/978-1-0716-0997-2_13)
43. **Lamidi S, Olalere R, Yekinni A and A Khairat** Design of Experiments (DOE): Applications and Benefits in Quality Control and Assurance. Industrial Engineering and Management. *IntechOpen.* 2024.  
<https://doi.org/10.5772/intechopen.113987>
44. **Seleiman MF, Al-Suhaibani N, Ali N, Akmal M, Alotaibi M, Refay Y, Dindaroglu T, Abdul-Wajid HH and ML Battaglia** Drought Stress Impacts on Plants and Different Approaches to Alleviate Its Adverse Effects. *Plants.* 2021; **10(2)**: 1-25.
45. **Yahaya MA and H Shimelis** Drought Stress in Sorghum: Mitigation Strategies, Breeding Methods and Technologies—A Review. *J. Agron. Crop. Sci.*, 2022; **208**: 127–142.



46. **Al-Rubaie A, Sh H and KDH Al-Jubouri** Response of growth and yield of industrial potatoes to soil improvement and spraying with tocopherol and trehalose under water stress. *Iraqi Journal of Agricultural Sciences*. 2023; **54(4)**: 963-978.
47. **Hu F, Zhang Y and J Guo** Effects of drought stress on photosynthetic physiological characteristics, leaf microstructure, and related gene expression of yellow horn. *Plant Signal Behav*. 2023; **18(1)**: 221-225.
48. **Benlioglu B and M Ozgen** *In vitro* selection of drought tolerant regenerates in durum wheat (*Triticum durum* desf.). *Applied Ecology and Environmental Research*. 2021; **19(3)**: 1813-1825.
49. **Toma RS** Minitubers production of four potato (*Solanum tuberosum* L.) cultivars by tissue culture technique. *Iraqi Journal of Agricultural Sciences*, 2022; **53(5)**: 1058-1066. <https://doi.org/10.36103/ijas.v53i5.1619>
50. **Baday SJS, Al-Jubori MT and MI Salih** *In vitro* responses of two barley varieties to Moringa extract and growth regulators under drought stress. *Asian J Agric*. 2025; **9(2)**: 358-367.
51. **Abed NY, Hadi BH, Hassan WA and KM Wuhaib** Growth traits and yield evaluation of Italian maize inbred lines by full diallel cross. *The Iraqi J. Agri. Sci*. 2017; **48(3)**: 773-781.
52. **Al-Jubori MT and MA Al-Shamari** Propagation and artificial seeds production from single node explants of maclura that cultured *in vitro*. *Iraqi Journal of Agricultural Sciences*, 2025; **56(Special)**: 190197. <https://doi.org/10.36103/tye6cd53>

