IMPROVEMENT OF GROWTH, PHYSIOLOGICAL AND BIOCHEMICAL TRAITS OF SUNFLOWER BY IAA AND BAP UNDER SALINITY STRESS *in vitro*

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ABSTRACT

This study was established to investigate the ability of the sunflower callus plant (*Helianthus annuus* L.) to tolerate salinity stress for two levels of NaCl (0 and 80 mM), three concentrations of IAA (0, 1.0, and 2.0 mg l^{-1}), and BAP (0, 1.0, and 2.0 mg l^{-1}) in lab conditions. Calli cultures were induced from the cotyledon of *H. annuus* L. cultured in an appropriate combination of growth regulators 2,4-D and kintein. The salinity results exhibited negative effects in most of the study indications, which can be revealed by a significant increase in sodium content (Na^+), hydrogen peroxide (H_2O_2) and malondialdehde content (MDA). While, the decrease in the study indications such as fresh weight (FW), dry weights (DW), relative water content (RWC), potassium content (K^+), sodium/ potassium ratio (Na^+/K^+), superoxide dismutase (SOD), and catalase (CAT) enzymes activity. The experiment revealed a positive effect for the exogenous growth regulators IAA and BAP in reducing the harmful effects of salinity stress on calli cultures. The nominated regulators succeeded in reducing the harmful effects of salinity stress, where the FW, DW, RFW, browning density (BI), RWC, K^+, Na^+/K^+, SOD, and CAT enzymes activity. However, the exogenous growth regulators reduced the negative effect for each of Na^+, H_2O_2, and MDA under the salinity stress.

Key words: Callus cultures, abiotic stress, relative water content, sodium chloride.

تحسين صفات النمو والفسلجية والكيموحيوية في زهرة الشمس باستعمال IAA و BAP تحت ظروف الشد الملحي خارج الجسم الحي.

صفا حامد خلف الخاطر شامل اسماعيل نعمة
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المستخلص

أجريت الدراسة الحالية بهدف اختبار قدرة مزارع كالس نبات *H. annuus L.* على تحمل اجهاد الملوحة لمستويين من ملح NaCl (0 و 80 mM) وثلاثة تراكيز من IAA (0 , 1.0 و 2.0 ملغم لتر$^{-1}$) وثلاثة تراكيز من BAP (0 , 1.0 و 2.0 ملغم لتر$^{-1}$) في ظروف المختبر. تم تنمية مزارع الكالس المُستحثة من الورقة الفلقية لنبات زهرة الشمس تحت توليفة مناسبة من منظمي النمو 2,4-D و Kintein. عكست نتائج الملوحة تأثيرات سلبية في معظم مؤشرات الدراسة, يظهر ذلك بوضوح في تسبب الملوحة في زيادة محتوى Na^+ و H_2O_2 و MDA وانخفاض لمؤشرات الدراسة المُتمثلة بالون الطري والوزن الجاف والوزن الطري النسبي والمحتوى المائي للكالس وأيون البوتاسيوم ونسبة K^+/Na^+ وفعالية إنزيم SOD وفعالية انزيم CAT. اظهرت نتائج التجربة التاثير الإيجابي للتطبيق الخارجي بمنظمي النمو IAA و BA للحد من التأثيرات الضارة لإجهاد الملوحة على مزارع الكالس, فقد نجحت المُنظمات آنفة الذكر في التخفيف من الآثار الناجمة عن الإجهاد الملحي, إذ ادى إلى زيادة الوزن الطري والوزن الجاف والوزن الطري النسبي وكثافة الإسمرار والمحتوى المائي للكالس ومستوى أيون البوتاسيوم ونسبة الصوديوم/ البوتاسيوم وفعالية انزيم SOD وفعالية انزيم CAT. في حين ادت الإضافة الخارجية لمنظمي النمو في الحد من الاثر السلبي لكل من آيون الصوديوم ومحتوى H_2O_2 و MDA تحت اجهاد الملوحة.

الكلمات المفتاحية: مزارع الكالس, الاجهاد اللاأحيائي, المحتوى المائي النسبي, كلوريد الصوديوم.

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INTRODUCTION

Sunflower is one of the essential strategic crops known for its benefits in nutrition and human body health because of its high content of unsaturated fatty acids, vitamins, minerals and dietary fiber. As well as containing some effective compounds such as flavonoids, tannins, alkaloids, phytic acid, essential oil and phenol compounds. It is derived from its therapeutic properties such as antioxidant, anti-inflammatory, anti-bacterial, anti-viral and anti-gout. Therefore, it has been widely used in traditional chinese remedies (16). Salinity stress is one of the drivers of climate change. It is one of the most dangerous abiotic stresses that affect plants. Especially in arid and semi-arid areas, due to increased salinity as a result of misuse of natural resources. *H. annuus* L. crop is classified as a salinity-sensitive plant, as the effect of salinity on the plant is not limited to a lack of irrigation water supplementary only but also to an imbalance in the ionic equilibrium within the plant tissues, which can, in turn, develop oxidative harm and hence damage the vital cellular molecules (21, 26). Due to the importance of this problem and its direct negative effects on agricultural production and to reduce the impact of this problem, specialists are working hard on developing research programs aiming at studying the efficiency of using plant growth regulators in increasing the tolerance of plant cells to the tension parameters resulting from the accumulated mineral elements as a result of salinity stress, as well as the development of new high salinity tolerance classes modern technologies, including in vitro cultivation technology (1). The technology of plant tissue culture is the most relevant for overcoming the problems caused by this stress due to the simplicity and accuracy by which it is characterized, as well as being economical and highly applicable. It contributes to facilitating and accelerating programs for obtaining tolerant or stress-resistant plants with an understanding of the mechanisms of tolerance or resistance and the accompanying physiological and biochemical changes, bypassing all restrictions resulting from the implementation of scientific experiments such as the planting season and the allocated regions of agricultural areas (7). Plant hormones play a significant role in the growth and development of plants by regulating many biological processes. Therefore, plant growth regulator treatment is one of the successful solutions to counter the various environmental stresses that plants are exposed to during their life cycle (9). The growth regulators IAA and BAP play a vital role in plant cell growth and development via increasing cell division and stimulating the biogenesis of chloroplasts under natural and saline conditions, as well as enhancing the antioxidant enzymatic system. They also contribute to the induction of gene expression responsible for enhancing plant cell tolerance to abiotic stress conditions, including salinity stress (12). This study was aimed to employ plant tissue culture technology to understand the mechanisms that can positively affect the tolerance of callus cultures of sunflower to salinity stress by testing the different concentrations of the plant growth regulators IAA and BAP and the interaction in affecting some growth, physiological, and biochemical traits of calli cultures of sunflower and to enhance tissue tolerance to salinity stress.

MATERIALS AND METHODS

A laboratory experiment was carried out in the plant tissue culture laboratory at Center of Desert Studies, University of Anbar. Using sakha-53 is one of the oilseeds. The report included the following stages:

Media preparation and seeds sterilization: The typical MS-type media was prepared according to the standards described by Murashige and Skoog (22). The media was prepared by adding 4.54 g l^{-1} and sucrose was added at 30 g. Then, the pH was adjust at 5.7 ± 0.1, and 7.0 g agar. The seeds were sterilized by NaOCl at 6% concentration for 10 min. One seed was placed per vial and incubated inside the growth chamber at 25 °C ± 1 illumination intensity of 1000 lux for 16 h and 8 h dark.

Callus induction: After germination, cotyledons were cultured in MS medium containing plant growth regulators represented by 2,4-D 1.0 mg l^{-1} with kinetin at a concentration of 0.75 mg l^{-1}. The cultures were then left in the growth chamber at 25 °C ± 1, with the same physical conditions, and

replanted every 35 days to obtain adequate callus culture.

Salinity stress parameters: The effect of the growth regulators IAA and BAP on the tolerance of calli cultures salinity stress was studied. The 8-weeks-old callus tissue was transferred from the sustaining medium to the salt-tolerant media prepared composed of MS medium of 4.54 g l^{-1}, sucrose of 30 g l^{-1}, and agar of 7.0 g l^{-1}. Which the prepared medium, included NaCl at 0 and 80 mM with BAP growth regulator, with concentrations of 0, 1.0 and 2.0 mg l^{-1}, taking the physical conditions that recommended by Yamakawa et al. (30), relating to the addition of IAA to maintain its stability in MS medium. The cultures were incubated in the growth chamber at 25˚C ±1 and 1000 lux for 16/8, represented by alternate light/dark. Results were recorded 30 days after culture.

Studied traits: Calculation of FW and DW: FW was measured after removing the media residue. The callus was then dried in an oven at 60˚C. Then the DW of the callus tissue was recorded.

RFW: The growth level was calculated based on the initial fresh weight of the cultured callus tissue (FW_i) and the final fresh weight of the callus tissue (FW_f) according to the following equation:

$$\mathbf{RFW} = \frac{\mathbf{FW_f - FWi}}{\mathbf{FWi}} \quad \mathbf{(5)}$$

RWC: RWC was calculated for calli cultures according to the method described by Karimi et al., (11), which is detailed in the following equation:

$$\mathbf{RWC}\ (\%) = \frac{\mathbf{FW - DW}}{\mathbf{TW - DW}} \times \mathbf{100}$$

Where:

FW= fresh weight of callus

DW= dried weight of callus

TW= Turgid weight of callus

Determination of Na^+ content: The Na^+ content of callus culture was determined according to the method described by Tendon (29), in which 200 mg of dried callus culture. The sample was placed in a 100 ml digestion tube. Digestion was carried out with perchloric and nitric acid with a ratio of 9:4. The powder was filtered into a standard 100 ml beaker, and the volume was filled with distilled water. The concentration of Na^+ in the sample was estimated using a flame photometer.

Determination of K^+ content

The K^+ content was estimated by taking 1.0 g of the dried sample and placing it in a digestion tube using the method of Semi-micro Kjeldahl (4) with the addition of 1.0 g of the $CuSo_4$ catalyst, after which 5 ml of concentrated sulfuric acid 98% was added. The digestion tubes were then placed on the heater to digest the sample. When the mixture became apparent, the samples were cooled. The solutions were diluted to 100 mL with distilled water. Then the potassium concentration was estimated by a flame photometer which gave the potassium concentration directly using the potassium standard curve.

Na^+/K^+ ratio: The ratio of Na^+/K^+ was calculated by dividing the Na^+ content in the callus culture by the K^+ content (20).

H_2O_2 content: The H_2O_2 content was estimated according to the method proven by AOAC (4). In 1.0 g of sliced sample was taken and smashed well with a small amount of distilled water. The mixture was then inserted into a 250 ml vial filled with extra distilled water. The 250ml vial was lifted for a short period to settle before taking 25 ml of it and put into a glass beaker, which was then filled with 250 ml of distilled water mixed with 10 ml of concentrated sulfuric acid gradually and then shaken carefully. The mixture was then flattened with 0.3 mm of potassium permanganate with well shaking until its color transformed into a pink color. The volume of used potassium permanganate was calculated at this step, and hydrogen peroxide was estimated.

MDA content: Thiobarbituric acid was determined according to the method mentioned by Love and Pearson (17). 1 g of the soft sample was taken and soaked for 2 min in 50 ml of distilled water. After then, the process included adding 5 ml of hydrochloric acid (4 N) solution to the soaked sample to reduce the pH to 1.5 and filling the remaining volume with 100 ml of distilled water. The mixture was then transferred to a 100 mL distillation flask, and 2 ml of paraffin oil and 1 g dry volume were added to regulate boiling and prevent fizzing. The distillation apparatus was connected and heated until 50 ml of the distillate was collected. The plank solution

was simultaneously prepared from distilled water. The absorbance was estimated using spectrophotometry at a length of 538 nm, and then TBA was calculated based on mg malonaldehyde/g^{-1}FW.

SOD enzyme activity: The method suggested by Marklund and Marklund (19) was used to estimate the activity of the SOD enzyme based on the enzyme's ability to inhibit the self-oxidation of pyrogallol in the presence of EDTA and pH 8.2. This method added 50 µl of plant extract and 200 µl of pyrakalol solution, and 2 ml of EDTA-Tris solution. The optical absorbance was then read at the wavelength of 420 nm.

CAT enzyme activity: The activity of the CAT enzyme was estimated according to the method described by Luhova et al. (18). Where 0.02 g of the sample was mashed with potassium phosphate solution (pH = 0.1M7.8, at a ratio of 1:2 w/v). The extract was then filtered with filter paper. Centrifugation was applied at 10,000 cycles for 30 minutes to estimate the activity of the CAT enzyme, where 200 microliters of the extract were taken and incubated with 1.0 ml of the mixture containing H_2O_2 (65mM) with neutral phosphate solution (pH = 60mM7.4) at 25 C for 4 minutes. The enzyme action was then stopped by adding 1.0 ml of ammonium molybdate (32.4 mM). The readings were taken to estimate the enzyme's activity at the wavelength (405nm).

Experimental design and statistical analysis The experiment was designed according to the complete random design (CRD) as factorial experiments with 10 replications for each treatment. The data were analyzed using the statistical analysis program (Genestat version 12). The relationship between the variables and the study features was also found by conducting the primary component analysis using PAST computer software.

RESULTS AND DISCUSSION

Growth traits: Some of the primary growth traits of callus *H. annuus* L. callus culture were studied. The results in Table 1 show a significant effects of the study factors on all growth traits. The untreated media with NaCl salt with high concentrations of IAA and BAP achieved the highest mean for FW and DW of callus, reaching 1035.3 and 67.67 mg, respectively. At the same time, the lowest value appeared from the interference of 80 mM of NaCl with a concentration of 0 mg l^{-1} for each IAA and BAP, which exhibited the least mean value for FW and DW of callus, reaching 263.3 and 16.77 mg, respectively. As for the RFW of the calli cultures, the results showed that the cultures grown in media not treated with NaCl salt with the high concentration of IAA and BAP achieved the highest mean value of 1.071, while the lowest mean (-0.473) due to the interference of the two treatments 80 mM of NaCl+0 mg l^{-1} IAA + 80 mg L^{-1} BAP. The BI represents one of the problems that negatively affect plant tissue growth (Figure 1). It could be noted from the results that the NaCl salt significantly increased the BI. Still, treatment with growth regulators caused a decrease in that density until it reached its lowest level when treating calli cultures grown in a media containing IAA and BAP at a concentration of 2.0 mg l^{-1} each was 2.667 (Table 1). Based on the results, the decreases in the FW and DW is an indication of the damage in calli cultures as a result of the exogenous of NaCl salt to a state of stress, as the increase in the level of salts caused a form of disturbances in the metabolic processes and the bio functions of cellular components (2), which negatively affected the FW and hence the DW. The reduction in BI with the increases of IAA concentration in tissue cultures is related to its role in protecting cell membranes from oxidative stress damage caused by salinity. It keeps the phenolic compounds isolated from the polyphenol oxidase enzyme in the plastids and protects them from oxidative damage. Therefore, the browning density of the callus tissue decreases.

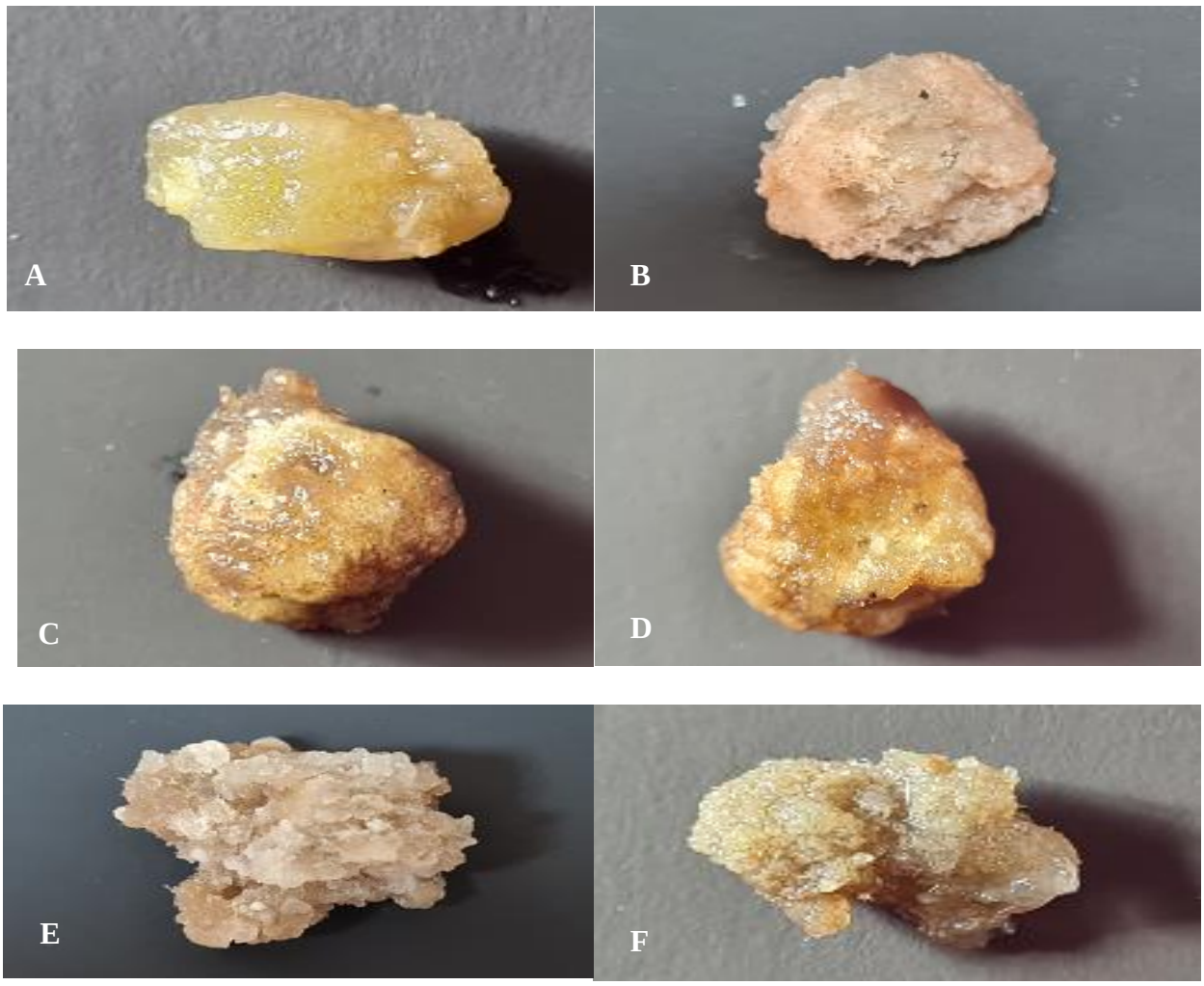

Figure 1. Induced calli cultures from cotyledons of *H. annuus* L. according to the following combinations (A) NaCl 0 mM×IAA 0 mg l^{-1}×BAP 1.0 mg l^{-1}(B) NaCl 0 mM×IAA 1.0 mg l^{-1}×BAP 2.0 mg l^{-1}(C) NaCl 0 mM×IAA 2.0 mg l^{-1}×BAP 0 mg l^{-1}(D) NaCl 80 mM×IAA 1.0 mg l^{-1}×BAP 1.0 mg l^{-1}(E) NaCl 80 mM×IAA 2.0 mg l^{-1}×BAP 2.0 mg l^{-1}(F) NaCl 80 mM×IAA 1.0 mg l^{-1}×BAP 0 mg l^{-1}

Physiological traits: The study of some physiological properties is a priority for preparing a report that explains the extent of their impact on growth features and the relationship between the two groups. The results in Table 2 show a significant differences between the various combinations in the RWC of callus cultures, as the media untreated with NaCl salt with a concentration of 2.0 mg l^{-1} for each of the growth regulators IAA and BAP achieved the highest average of 88.11%. In contrast, the lowest average value appeared where the interference of 80 mM NaCl with 0 mg l^{-1} of IAA and BAP existed, with the lowest mean value of the trait being 60.41%. The results also showed that the study factors significantly affected the content of callus tissue of Na^+, as the media treated with 80 mM of NaCl salt with the untreated with IAA and BAP achieved the highest average value of 27.667 mg g^{-1} dry weight. In contrast, the media without treatment with NaCl salt reached a concentration of 2.0 mg l^{-1} of IAA and 1.0 mg l^{-1} of BAP, the lowest average, which was 1.233 mg g^{-1} dry weight. The results also showed a significant difference for the various combinations on the effect of the K^+ content in callus cultures. Untreated media with NaCl salt with a high concentration of IAA and BAP growth regulators achieved the highest average value of 19.267 mg g^{-1} dry weight, while the concentration of 80 mM of K^+. NaCl salt with 0 mg l^{-1} for each IAA and BAP achieved the lowest mean value of 16.400 mg g^{-1} dry weight. The results revealed that the ratio of Na^+/K^+ was significantly affected by the treatment with the various combinations, as the concentration of 80 mM of NaCl salt with the comparison treatment of IAA and BAP achieved the highest average value of 1.6869. In contrast, the lowest average reached 0.0661 with the comparison

treatment of NaCl salt with 2.0 mg l^{-1} of IAA and 1.0 mg l^{-1} of BAP. The accumulation of harmful ions in the plant cell due to salinity stress caused by treatment with NaCl salt results in a decrease in its osmotic ability and thus a reduction in the absorption of water by the callus tissue from the nutrient medium, and this explains the reduction in RWC with the increase of accumulation of salinity in the plant tissue. At the same time, treatment with plant growth regulators (IAA and BAP) contributed to stabilizing metabolic processes disturbed by salinity. Ashoori et al. (3) study referred that salinity disrupts the bio pathways responsible for building chlorophyll in the plant cell, as some plant growth regulators have roles that qualify them to protect plant cells from the impact of various environmental stresses, including salt stress, as they caused reduce the harmful effects of salinity in callus cultures by decreasing the accumulation of Na^+ and increasing the accumulation of K^+ (10). These results indicated the contribution of some plant growth regulators in protecting the plant cell from the negative impact of salinity stress by reducing the accumulation of Na^+ and increasing K^+ accumulation (Table 2). This result similier Koutoua et al., (15) in callus of *Triticum durum*.

Table 1. Three-way interaction means comparison for NaCl, IAA and BAP related to growth traits of *H. annuus* L. calli cultures induced from cotyledon leaves after 30 days of cultured in MS media

NaCl (mM)	IAA (mg l^{-1})	BAP (mg l^{-1})	FW (mg)	DW (mg)	RFW	BI
0	0	0	678.3	44.34	0.357	1.333
		1.0	792.3	51.79	0.585	1.333
		2.0	781.3	51.07	0.563	2.333
	1.0	0	636.0	41.57	0.272	2.000
		1.0	750.3	49.04	0.501	2.667
		2.0	812.3	53.09	0.625	1.667
	2.0	0	700.0	45.75	0.400	3.000
		1.0	893.3	58.39	0.787	1.667
		2.0	1035.3	67.67	1.071	2.667
80	0	0	263.3	16.77	- 0.473	5.000
		1.0	440.7	28.07	- 0.119	4.667
		2.0	490.3	31.23	- 0.019	4.667
	1.0	0	517.3	32.95	0.035	4.000
		1.0	513.0	32.68	0.026	3.667
		2.0	518.0	32.69	0.039	3.000
	2.0	0	516.0	32.87	0.032	3.333
		1.0	614.7	39.15	0.229	3.333
		2.0	534.3	34.03	0.069	2.667
L.S.D < 0.05			71.00	4.644	0.1420	0.9148

Table 2. Three-way interaction means comparison for NaCl, IAA and BAP related to physiological traits of *H. annuus* L. calli cultures induced from cotyledon leaves after 30 days of cultured in MS media

NaCl (mM)	IAA (mg l^{-1})	BAP (mg l^{-1})	RWC (%)	Na^+ (mg g^{-1} DW)	K^+ (mg g^{-1} DW)	Na^+/K^+
0	0	0	83.32	2.067	17.167	0.1205
		1.0	84.54	1.967	17.800	0.1105
		2.0	85.20	1.933	17.967	0.1077
	1.0	0	84.80	1.933	17.833	0.1085
		1.0	86.81	1.667	17.533	0.0952
		2.0	86.64	1.333	17.933	0.0744
	2.0	0	86.48	1.500	18.067	0.0830
		1.0	88.10	1.233	18.667	0.0661
		2.0	88.11	1.900	19.267	0.0986
80	0	0	60.41	27.667	16.400	1.6869
		1.0	63.39	26.000	16.733	1.5536
		2.0	67.90	23.333	16.700	1.3978
	1.0	0	71.12	24.667	16.933	1.4573
		1.0	70.60	21.667	17.200	1.2617
		2.0	68.83	15.333	16.933	0.9061
	2.0	0	71.82	15.667	17.133	0.9146
		1.0	75.77	12.667	17.100	0.7409
		2.0	74.03	14.000	16.733	0.8369
L.S.D < 0.05			2.332	1.0432	0.5352	0.07410

Biochemical traits: The traits of the biochemical study are essential in determining the extent of changes that occur to it as a result of different treatments and their reflection on the physiological traits (25). It could be noted from the results that there is a significant effects of the treatments on the H_2O_2 content, as the level of 80 mM with NaCl salt not treated with plant growth regulators achieved the highest mean for the trait achieved 112.40 µg g^{-1}. In contrast, the lowest mean value was 55.77 µg g^{-1} when the nutrient medium was not included in NaCl salt with 2.0 mg l^{-1} of IAA and 1.0 mg l^{-1} of BAP. A significant effect was also showed in the content of callus tissue on MDA, as treatment with 80 mM of NaCl salt with no treatment with growth regulators achieved a high mean of 3.267 mg g-1 fresh weight. In contrast, the comparison treatment of NaCl salt with the concentrations of 2.0 mg l^{-1} of IAA and 1.0 and 2.0 mg l^{-1} of BAP achieved the lowest Average for the trait, which was 1.467 mg g^{-1} fresh weight. The activity of the SOD enzyme was significantly affected by the different treatments, as the media untreated with NaCl salt with high concentrations of IAA and BAP achieved the highest average value of 138.67 IU min^{-1} mg^{-1}. In contrast, the level was completed by 80 mmol of NaCl salt with 0 mg l^{-1} for each IAA and BAP. The lowest average value was 76.67 IU min^{-1} mg^{-1}. The different treatments also significantly affected the activity of the CAT enzyme. The media untreated with NaCl salt with a concentration of 2.0 mg l^{-1} of IAA and aconcentration of 1.0 mg l^{-1} of BAP achieved the highest mean of 183.7 mmol min^{-1} mg^{-1}. In comparison, the lowest recorded Average at 80 mM of NaCl salt with concentrations of 0 mg l^{-1} IAA+2.0 mg l^{-1} of BAP was 117.7 mmol min^{-1} mg^{-1}. The salinity stress stimulated the formation of free radical species in the plant cell represented by H_2O_2. These factors caused damage to the cellular molecules responsible for promoting plant growth, which led to a decrease in the development of callus cultures. The increase in MDA content in the plant cell from the effect of NaCl salt treatment is an indicator of the extent of damage to cell membranes due to the increase in reactive oxygen species up to oxidative stress, and this was indicated by previous reports (6, 28), and this is directly related to the extent of stress applied to the implanted tissue. Maintaining the stability of biomembranes and protecting them from the negative impact of oxidation caused by environmental stress factors is the primary goal to overcome this sensitive stage, as membrane damage results in an increases in electrolytic exudation, which could be caused a migration of the essential ions to the plant cell, foremost of which is K^+. The cell loses its main growth components (20, 28). It increases the callus tissue content of H_2O_2 as one of the types of ROS confronted by the cell's defence systems, including the antioxidant enzyme system. SOD enzyme is considered the primary enzyme that reduces the risk of oxidative factors in the cell due to its work on breaking H_2O_2 into H_2O+O^{-2} (8, 23, 24).Treatment with plant hormones, whether IAA or BAP, plays a vital role in reducing the severity of environmental stresses, including salinity stress (2), as it works to scavenge free radicals, including H_2O_2, which is caused by the negative effect of oxidative stress.

Table 3. Three-way interaction means comparison for NaCl, IAA and BAP related to biochemical traits of *H. annuus* L. calli cultures induced from cotyledon leaves after 30 days of cultured in MS media

NaCl (mM)	IAA (mg l^{-1})	BAP (mg l^{-1})	H_2O_2 µg g^{-1}	MDA mg g^{-1} FW	SOD IU min^{-1} mg^{-1}	CAT mmol min^{-1}
0	0	0	80.57	2.467	107.33	138.0
		1.0	81.87	2.533	103.00	145.7
		2.0	84.33	2.667	113.00	140.0
	1.0	0	75.13	2.100	111.33	141.3
		1.0	81.97	2.633	103.33	130.3
		2.0	72.77	1.967	122.00	148.3
	2.0	0	68.97	1.733	133.00	153.7
		1.0	55.77	1.467	138.00	183.7
		2.0	56.23	1.467	138.67	181.7
80	0	0	112.40	3.267	76.67	121.7
		1.0	94.97	3.100	89.00	123.7
		2.0	95.20	3.100	90.33	117.7
	1.0	0	89.13	2.967	94.33	126.7
		1.0	86.37	2.533	105.67	128.0
		2.0	85.90	2.467	95.33	128.0
	2.0	0	87.13	2.700	98.00	128.0
		1.0	86.37	2.567	98.33	124.0
		2.0	87.60	2.600	110.67	136.0
L.S.D < 0.05			10.811	0.3125	10.650	13.57

This is due to the role of these regulators in increasing the effectiveness of some antioxidant systems in the plant cell, including the antioxidant enzyme system, and the CAT enzyme is one of the leading enzymes in this group (9). This result is similar to what was found by Khalid and Aftab (13) in *Solanum tuberosum* L., Khuder and Al-Taei (14) in *Triticum aestivum* L., Yasir et al. (31) in *Fragaria×ananassa* Duch in vitro, Zaidan (32) in calli cultures of *Triticum aestivum* L., and Zulfiqar et al., (33) in *H. annuus* L.

Principal component analysis

Principal component analysis (PCA) was performed on the averages of the results to examine the effect of multiple treatments on certain important traits of *H. annuus* L. The principal component (PCA) revealed the maximum contribution among all components, accounting for 99.22% of the total variance in the data set according to the analysis (Fig. 2). The successful distribution among all treatments gave a clear indication that IAA and BAP positively affect *H. annuus* L. under saline and non-saline growth (Fig. 2). Saline treatment (80 mM NaCl) without IAA or BAP showed adverse effects on FW, DW, RFW, RWC, K^+, SOD and CAT. While the exogenous of both IAA and BAP improved these parameters, reducing the BI density and the content of Na^+, H_2O_2 and MDA.

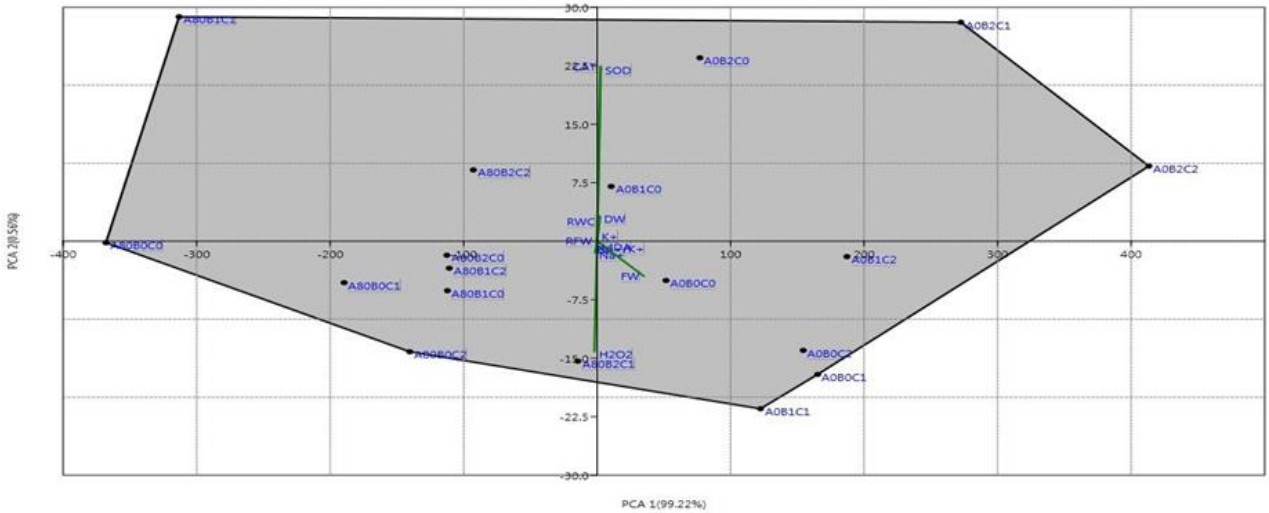

Figure 2. Principal component analysis (PCA) of different studies (A: NaCl, B: IAA, and C: BAP) in callus culture of *H. annuus* L

CONCLUSION

The growth regulators IAA and BAP contributed to the improvement of most of the growth, physiological and biochemical properties of tissue cultures grown under stress or normal conditions. The combination of the plant growth regulators had the most prominent role in improving the growth, physiological and biochemical traits, especially the combination of the high concentration of IAA growth regulator and the middle concentration of BAP (1.0 mg l^{-1}). It could be indicate the futility of resorting to the high concentration of BAP. The results of salinity were evidence of the possibility of treatment with IAA or BA or both to reduce the harmful effects of salinity stress on sunflower crop ex vivo since the nominated regulators have successfully mitigated the impact of salt stress by reducing the accumulation of Na^+ which restores the mineral balance throughincreased K^+, and by promoting the antioxidant enzymes SOD and CAT that reduce H_2O_2 oxidative stress, and maintain osmotic modulation by osmotic regulation. Both plant regulators showed similar effects on growth traits and improved physiological and biochemical traits of sunflower callus. However, we found that IAA showed a more remarkable ability to reduce the accumulation of Na^+, H_2O_2 and MDA content and increase SOD and CAT activity by BAP.

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