EFFECTS OF GAMMA RAYS AND SODIUM CHLORIDE ON GROWTH AND CELLULAR CONSTITUENTS OF SUNFLOWER (HELIANTHUS ANNUUS L.) CALLUS CULTURES

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SUMMARY: The effect of gamma rays and NaCl on growth and cellular contents of soluble carbohydrates, protein and nucleic acids in Helianthus annuus L. callus were investigated. Optimal callus initiation was attained in stem segments cultured in MS medium enriched with 0.05 mg/1 2,4-dichloro phenoxyacetic acid (2,4-D) and 0.01 mg/1 N^{-6} furfurylamino purine (kinetin). Radio sensitivity, based on fresh weight changes, was determined following exposure of the calli to different doses of gamma rays. The LD50 was calculated and it was equal to 2.8 krad. Inclusion of NaCl in the medium caused a significant reduction in callus fresh weight. In general, the cellular contents of protein, soluble carbohydrates and ribonucleic acid (RNA) were reduced, while deoxyribonucleic acid (DNA) increased at 2% NaCl level. There is a significant increase in protein, carbohydrates and DNA, while a significant reduction in RNA content was observed. The role of such information in breeding for salt tolerant sunflower following physical mutagenesis in vitro is outlined.

Key Words: Helianthus annuus L., gamma rays, salt tolerance, mutation breeding, callus cultures.

INTRODUCTION

The sunflower (Helianthus annuus L.) represents one of the most important oil crops in Iraq. It is widely cultivated in the northern regions under dry land farming system. Salinity is one of the major problems that faces the farmers all over the world. According to Mass and Hoffmann (14), it is estimated that one third of the irrigated land in the world is affected by high salinity. Iraq is one of the countries that have been highly affected by salinity, and a large area of agricultural lands are now rated as unproductive as a result of salinity. The central and southern regions of Iraq have been classified as moderate to highly saline soils (2), with chloride and sulphate salts of Na^{+}, Ca^{++}, Mg^{++} as the major soluble salts.

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The overall tolerance to salinity differs among different plants. Sunflower is classified as moderately sensitive to salinity. Therefore, much work is needed to improve the ability of sunflower to develop under such stress. During the past few years, and as a result of the advancement of the tissue culture technology, success has been achieved in selection of cell lines that showed tolerance to high salinity, from which plants were successfully regenerated. Such plants included Oryza sativa L. (9,19), Citrus sinensis and C. aurantium (12) and Medicago sativa (24). In view of the economic importance of sunflower, the present work was conducted to determine the effects of gamma rays and NaCl on some cellular contents of sunflower callus cultures, as the first step towards using such technique for production of salinity tolerant sunflower.

Table 1: Effect of 2, 4-D and kinetin on callus initiation from stem segments of sunflower (FAW genotype). Each number represent mean fresh weight of 10 replicates.

2, 4-D mg/1 Conc	mean fresh weigt (mg) (B)					Mean
(A)	0.0	0.01	0.1	1.0	10.0	
0.0	880.00	575.95	596.72	938.43	646.87	722.19
0.01	1051.60	695.50	395.25	764.00	550.00	691.27
0.05	577.00	990.71	477.39	749.33	1151.83	789.27
0.5	97.30	159.00	138.50	176.00	284.83	171.12
5.0	53.50	49.00	63.00	63.50	66.50	60.30
Mean	531.88	494.03	329.97	538.25	540.00	

LSD (A) at 5% = 261.17
LSD (AB) at 5% = 116.8

MATERIALS AND METHODS

Seeds of Helianthus annuus L. genotype FAW (a mutant which was developed in our center following gamma irradiation) were surface sterilized, and their embryos cultured on minimal organics medium. Following germination, stem segments, measuring, 1 cm long, were excised and cultured on Murashige and Skoog (15) medium enriched with various concentrations of 2, 4-D (0-5 mg/1) and kinetin (0-10 mg/1) to stimulate callus initiation. The resulting callus was then propagated on the selected medium by sub culturing twice until the desired amount of callus sufficient for the irradiation experiment was attained. The callus was exposed to different doses of gamma rays from a Co^{-60} source to determine its radio sensitivity and to increase the genetic variations. The doses used in this experiment were 0.5, 1.0, 1.5 and 3.0 krad, in addition to the control. Following determination of the LD50 (i.e. the dose that kills about 50% of the cells) which is suitable for mutation selection, the callus cultures that received this dose were further propagated twice and sub-cultured on nutrient medium enriched with 0, 0.5, 1.0, 2.0 and 3.0% NaCl as the salinity stress agent. The electrical conductivities (EC) of the media following NaCl additions were 5.88, 14.57, 23.52, 38.78 and 52.0 dS/m. The callus cultures were allowed to grow on such media (containing NaCl) for 60 days in the culture room at 27±1°C in the dark. Fresh weight was determined to evaluate the callus growth. The cellular contents of protein, soluble carbohydrates and nucleic acids were determined and adapted as parameters to assess the effects of the stress agents on the cultured cells. Protein contents were measured by the Folin method (13), and the soluble carbohydrates by the Phenol-Sulphuric acid method as described by Herbert *et al.* (10). Total nucleic acids were determined following the method of Cherry (8) which is based on the difference in the absorption at 260 and 290 nm. DNA was measured according to Burton (7) depending on the optical density at 600 nm. RNA was then calculated from the difference between the total nucleic acids and DNA content. Standard curves for carbohydrates, protein, DNA and RNA were prepared with similarly treated glucose, bovine serum albumin, calf thymus gland and yeast nucleic acids, respectively.

RESULTS AND DISCUSSION

Following 4 weeks of incubation of stem segments on nutrient medium enriched with different concentrations of 2,4-D and kinetin, callus initiation was observed in all test combinations. However, the amount of callus initiated varied at different hormonal supplementations (Table 1). The best callus was initiated in nutrient medium enriched with 0.05 mg/1 2,4-D and 0.01 mg/1 kinetin (Table 1). Although higher fresh weight was attained in some other treatments, the callus in such treatments was compact, necrotic and showed a differentiation of some roots. The general appearance of the callus in the selected treatment had creamy to white color, and no necrotic regions were observed. Callus propagation was achieved by subsequent subculture on nutrient medium enriched with 2,4-D (0.05 mg/1) and kinetin (0.01 mg/1). The initiation of callus

Table 2: Effect of gamma rays on callus fresh weight.

Dose (Krad)	Fresh weight (mg)
0.0	2.90a
0.5	2.24b
1.0	1.81c
1.5	2.30a
3.0	1.45c

Numbers followed by the same letter within the column are not significantly different according to Duncan's test at 0.05 level.

Table 3: Effect of NaCl on callus fresh weight, carbohydrate and protein in sunflower (FAW genotype).

NaCl conc. (%)	callus fresh weight (mg)	protein (μg/mg)	carbonhydrates (μg/mg)
0.0	1337 a	1.263 a	1.295 c
0.5	680 b	1.182 b	1.710 b
1.0	422 c	0.582 c	2.245 a
2.0	347 c	1.021 b	1.860 d
3.0	382 c	0.541 c	0.975 e

Numbers followed by the same letter within the column are not significantly different according to Duncan's test at 0.05 level.

cultures from stem segments of sunflower has been reported on similar hormonal supplementation (1).

The effect of gamma rays on callus fresh weight is shown in Table 2. There was a significant reduction in callus fresh weight with increased doses of irradiation. The LD50 was 2.8 krad. A similar reduction in callus fresh weight as a result of gamma irradiation has been reported (4,11,16). This reduction in fresh weight may be caused by the reduced amount of endogenous growth regulators, especially the cytokines, as a result of break down, or lack of synthesis, due to irradiation.

The effect of NaCl on callus fresh weight is shown in Table 3. Sodium chloride caused a significant reduction in callus fresh weight. The higher the NaCl concentration in the culture medium caused the higher the reduction in callus fresh weight. This is in agreement with the results reported by several investigators (6, 22, 24). The reduction in callus fresh weight might be a result of reduced water availability in the culture medium due to increased NaCl concentrations. The cellular contents of protein, soluble carbohydrates and nucleic acids were also affected by the presence of NaCl in the culture medium. Protein contents were significantly reduced at all concentrations of NaCl tested, with the maximum reduction being obtained at the highest NaCl concentration (Table 3). A comparable level of reduction in protein content was also observed at the 1% NaCl level which was not significantly different from that associated with the 3% level. There was a sudden rise in protein content at the 2% NaCl level, which may be possibly caused by the presence of NaCl tolerant cells within the callus that received such treatment. The contents of carbohydrates were also significantly reduced at the highest NaCl concentration (Table 3). However, there was a significant increase at the intermediate level. At 1% NaCl level, the highest amount of soluble carbohydrates was detected, which may also suggest the presence of tolerant cells. Results presented in Table 4 indicate no significant changes in total nucleic acid contents in different NaCl concentrations, however, a non-significant reduction was observed at the 1 and 2% NaCl level. On the other hand, the DNA contents were increased with the increased NaCl concentration, while RNA decreased. A significant decrease in RNA was observed at the 2% NaCl, while a significant increase in DNA content was detected at the same level. In fact, at the 2% NaCl level, a significant increase in protein, carbohydrates and DNA was observed while significant reduction in RNA was attained. This suggests that this level of NaCl may be the appropriate level for selection of NaCl tolerant cell line.

The reduction in protein contents noticed in this investigation is in accordance with the results of other investigators (18, 20, 22, 23). High concentration of NaCl in the culture medium may affect protein synthesis through inhibition of some enzymes that regulates the process of protein synthesis, especially nitrate reductase (17). This reduction may also result from reduced RNA content, which is necessary for protein synthesis (3). Carbohydrate contents were generally reduced with increased NaCl concentrations. However, their increase at specific NaCl concentrations may have resulted from the hydrolysis of protein into simpler compounds. The reduction in soluble carbohydrate contents in NaCl containing media probably resulted from the conversion of the insoluble into soluble

Table 4: Effect of NaCl on nucleic acid contents (μg/mg) of sunflower callus.

NaCl conc. (%)	Total nucleic acids	DNA	RNA
0.0	0.417 (1.345)	0.107 (0.816)	0.310 (1.317)
0.5	0.414 (1.827)	0.083 (2.590)	0.358 (2.128)
1.0	0.213 (2.75)	0.112 (0.445)	0.101 (2.212)
2.0	0.223 (2.45)	0.172 (4.008)*	0.051 (3.057)*
3.0	0.458 (2.168)	0.116 (0.148)	0.342 (1.858)

*: significant at the 5% level
Numbers in parenthesis represent calculated t value.

$$t = \frac{\bar{X}}{\frac{S}{\sqrt{n}}}$$

carbohydrates such as starch. These observations are in agreement with the findings of others (3, 21, 25).

Presence of NaCl in the culture medium also reduced DNA and RNA contents. The process of DNA synthesis is connected to protein synthesis. Therefore, the reduction in RNA content will ultimately reduce the protein content, since RNA is required for the process of protein synthesis through transferring the amino acids into protein synthesis centers (26). It is also possible that NaCl may also hydrolyze the ribosomes which are necessary for protein synthesis, or activate RNase that catalyzes RNA hydrolysis, and subsequently reduces protein synthesis.

This investigation showed that irradiation and NaCl has drastic effects on callus fresh weight and the cellular constituents of sunflower. Information gained from this study were further used in currently going program aiming towards developing salt tolerant cell lines through selection in vitro. Such cell lines will be further used for regeneration of possibly salt tolerant sunflower plants.

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