Egyptian Journal of Botany

http://ejbo.journals.ekb.eg/

Changes in the Profiling of Fatty Acids of *Glycine max* L. (Soybean) Callus after Mutagen Treatments

Rehab M. Hafez[1]#, Abdallah A.Y. Mohammed[2], Abd El-Rahman M. Abd El-Naby[2], Ali E.A. Tolba[2], Eslam Y.M. Khalifa[2], Hamed M. Hamed[2], Mohammed M.K. Abdullah[2], Mohammed M. F. Ahmed[2], Mohammed S. Hekal[2], Dalia H.A. Ali[2]
[1]Botany and Microbiology Department, Faculty of Science, Cairo University, Giza 12613, Egypt; [2]Chemistry Department, Faculty of Science, Cairo University, Giza 12613, Egypt.

THE AIM of present study was to produce healthy friable calli from soybean cultivar Giza 111 and studying the effect of sodium azide (SA) and ethidium bromide (EtBr) mutagens on its fatty acids (FAs) profile. Seeds were surface sterilized with clorox and ethanol, then cultured on solid Murashige and Skoog medium (MS) to produce 14-days-old *in vitro* plantlets. Callus induction from hypocotyl (H), cotyledon (C), epicotyl (E) and leaf (L) explants were tested using different combinations of 2,4-dichlorophenoxy acetic acids (2,4-D) and N6-benzyladenine (BA). M4 (MS+ 0.5mg/L BA) medium was found to be the supreme medium for callus proliferation. Both H-and E-explants were the best explants for this proliferation. Growth dynamics of H- and E-calli was established. The best harvesting time for both calli was the 4th week. H-calli were the most rapidly growing one. The concentrations 0.5, 1.0 and 1.5mM of each mutagen with two soaking times (½ and 1hr) were used for the treatment of H-calli. After mutagenic treatment, treated-H-calli were cultured on callus induction media. A month later, FAs profiling of mutated and control H-calli were performed using gas liquid chromatography (GLC). The Fw pattern of SA and EtBr-treated-H-calli were similar to the control one but with lower values. Saturated (SFAs) and unsaturated-fatty acids (UFAs) contents were raised up by increasing the concentrations of SA and time of incubation. In EtBr-treated-H-calli, marked elevations of SFAs over UFAs were recorded exhibiting a switch in their balance by increasing concentrations and soaking times.

Keywords: Calli, 2,4-dichlorophenoxy acetic acids, Fatty acids, *Glycine max* L., N6-benzyladenine, Mutagens.

Introduction

Glycine max (L.) Merr. (soybean), is an important seed legume from the family Fabaceae. The ability of soybean to withstand various climatic zones and environmental conditions worldwide makes it one of the most precious oil seed crops (Joyner et al., 2010).

Soybean possess a very high nutritional value with a relatively high protein (39-45%) and oil contents (20-30%) (Abolude et al., 2012). The soybean oil contains SFAs and UFAs represented as 53% α-linolenic acid, 23% oleic acid, 12% palmitic acid, 8% linoleic acid and 4% stearic acid (Olguin et al., 2003; Lee et al., 2007; Nikolić et al., 2008). Recently, the analysis of FAs received great attention for their diverse potential values in nutritional and health implications (Sahena et al., 2009). Fractionation of oil in terms of its FA composition is very important for producing products with physical or nutritional properties of interest to the food industry (Soares et al., 2007) and with pharmaceutical properties for health care (Napier & Michaelson, 2001; Shanab et al., 2018).

The United States of America, Brazil, Argentina, China and India are the largest producers of this crop (Masuda & Goldsmith, 2009) and about 85% of the total soybean production goes for oil extraction (Karthika & Lakshmi, 2006). It is expected that world-wide production of soybean will rise from 210.9 million metric tons (in 2009) to 311.1 million metric tons by 2020 (Masuda & Goldsmith, 2009; Soystat, 2010). But About 37% of the crop yield was lost by facing incidences of several diseases and adverse environmental conditions (Slater et al., 2008) causing economic disasters and loss of millions dollars in seed-oil manufacturing.Therefore, sustainable crop improvement is required to overcome those obstacles and to boost the production of the important FAs in seed-oil plants.

Tissue culture is the rachis of techniques leading to successful mutated cells, calli, organs or even whole plants, depending on the identification of more effective and efficient mutagens, as well as on optimized tissue culture conditions (Srivastava et al., 1995).

The prospective of induced mutations, either by physical and chemical mutagens, in boosting the genetic diversity in soybean to improve its economic va1ue is now applied by several investigators (Khan & Tyagi, 2013). Most of the amended varieties were derived from treated seeds and seedlings searching for the impact of mutagens on survival, yield, germination and morphological traits (Carroll et al., 1986; Karthika & Lakshmi, 2006; Patil & Wakode, 2011; Gobinath & Pavadai, 2015; Khan & Tyagi, 2017; Sagel et al., 2017). Other mutants were induced for improving their seed oil compositions (Wilcox et al., 1984; Rahman et al., 1994, 1995; Patil et al., 2007; Hanafiah et al., 2010). To our knowledge, no reports were recorded focusing on induced mutation in soybean calli.

Therefore, the current research article aimed to inducing calli from soybean different explants with variable amount of growth hormones. Then, to select the best calli-derived explant for accessing the impact of SA and EtBr on their FAs portray.

Materials and Methods

The media components and phytohormones were tissue culture grade. All solvents used for chromatographic purposes were HPLC grade.

2,4-D and BA (Sigma-Aldrich) were prepared as separate stock solutions (1mg/ml).

Dry seeds of *Glycine max* (L.) (soybean) cv. Giza 111 were kindly provided from Legume Department, Horticulture Research Institute, Agriculture Research Center, Giza, Egypt. The seeds were used as initial plant material (Fig 1).

Fig.1. Dry seeds of *Glycine max* (L.) cv. Giza 111.

Seeds sterilization

Homogenous seeds were surface sterilized according to the method described by Kotchoni et al. (2012). The seeds were washed with soapy-water for 10min then rinsed 3-4 times with running tap water for 15min to remove the soap. The seeds were surface sterilized by immersing in a mixture of 50% (v/v) Clorox® (containing 2.625% sodium hypochlorite, NaOCl) and 0.1% (v/v) Triton x-100 for 30min with stirring and then rinsed with 70% ethanol for not more than 1min. The alcohol was decanted, and the seeds were washed thoroughly (4-5 times) with sterile double-distilled water (Hamilton, Laboratory glass L.T.D.) under a sterile air laminar flow hood (Bioair, Euroclone, Siiano-Italy).

Seeds germination

For germination, ten sterilized seeds were aseptically cultured in 350ml screw-capped glass jars; each jar contains 50ml of germination medium (Fig. 2). A number of fourteen jars were prepared as replicates. Germination medium contains inorganic salts and vitamins of MS medium (Caisson labs, Murashige & Skoog, 1962) supplemented with 30g/L sucrose, 100mg/L myo-inositol and 8g/L agar. The pH of the medium was adjusted to 5.8 before autoclaving at 1.21kg cm^{-2} for 20min. All cultures were maintained in temperature-controlled growth room at constant temperature (25±2ºC)

681

CHANGES IN THE PROFILING OF FATTY ACIDSOF *GLYCINE MAX* L. (SOYBEAN) ...

with 16hr photoperiod under cool-white fluorescent light (1000 Lux irradiance) for 14 days. Percentage of contamination and % germination were recorded at the end of the experiment.

Fig. 2. Sterilized seeds cultured on germination medium.

Explants and callus induction

Callus cultures of soybean were initiated by placing excised sterile H, C, E and L-explants of 14 day-old aseptically growing seedlings in 200ml screw-capped glass jars containing 25ml callus induction media. The size of the explants was 5-10mm length for H- and E-explants, 8-10mm with 2-3mm thickness for C-explants

and L-explants with central midrib (10mm²) (Fig. 3). Ten explants per jar for H- and E-explants and seven explants per jar for C- and L-explants were cultured. Triplicates were prepared for each explant in each treatment. Callus induction media consists of MS medium supplemented with different concentrations of 2,4-D and/or BA as follows:

      M1 (MS+ 1mg/L 2,4-D)
      M2 (MS+ 2mg/L 2,4-D)
      M3 (MS+ 3mg/L 2,4-D)
      M4 (MS+ 0.5mg/L BA)
      M5 (MS+ 1mg/L 2,4-D+ 0.5mg/L BA)
      M6 (MS+ 2mg/L 2,4-D+ 0.5mg/L BA)
      M7 (MS+ 3mg/L 2,4-D+ 0.5mg/L BA)

Hormone-free MS medium (MS) was used as control.

All cultures were maintained in temperature-controlled growth room at constant temperature (25±2ºC) with 16hr photoperiod under cool-white fluorescent light (1000 Lux irradiance) for 2 weeks.

The growth of callus and any morphogenetic responses was determined at the end of the 1st and the 2nd week. The evaluation of the type and concentration of the growth regulators that gave highest, healthy callus proliferation from different explants were detected. The best explant-derived callus and the best callus induction medium will be selected after the end of the 2nd week for performing callus stocks.
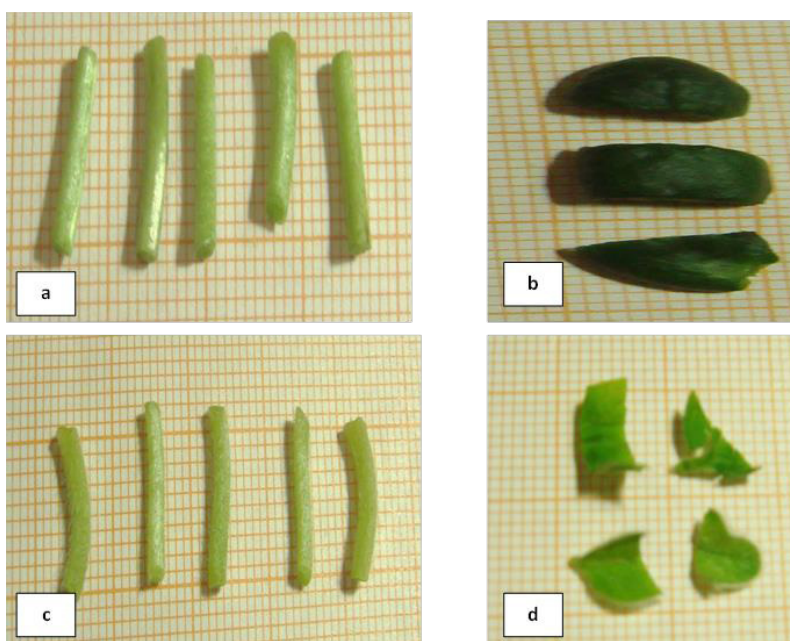
Fig. 3. The different explants used for callus proliferation; a) H-explants, b) C-explants, c) E-explants and d) L-explants.

Callus stocks

Subsequent subcultures of the best explant on the best calli proliferation medium were performed every two weeks. The cultures were maintained in temperature-controlled growth room at constant temperature (25±2ºC) with 16hr photoperiod under cool-white fluorescent light (1000 Lux irradiance). These subcultures were performed to obtain large quantities of healthy callus for further studies.

Growth dynamics of the best callus

The healthy calli were subcultured, while brown ones were discarded. Selected calli pieces from callus stock of the best explant were used to follow their growth pattern. In laminar flow, three inoculates of calli with average Fw of 0.5g were transferred (inoculum/jar) to reweighed jars containing the best callus proliferation medium. All cultures were maintained in temperature-controlled growth room at constant temperature (25±2ºC) with 16hr photoperiod under cool-white fluorescent light (1000 Lux irradiance).

For characterizing the growth of calli and detecting the best harvesting time, fresh weight (Fw, g/explant), dry weight (Dw, g/explant) and growth rate (GR) (g/day) were determined every week for 5 weeks according to Dung et al. (1981), where Fw was determined after cleaning the callus from agar, Dw was determined after drying the callus in an oven at 60ºC until constant weight and GR was calculated as follow:

$$GR= Fw_{final} - Fw_{first}/time\ in\ days$$

Mutagenesis

The mutagens used to induce mutation in soybean callus were SA and EtBr. The concentrations 0.5, 1 and 1.5mM of each mutagen were used for the treatment of callus. Non-treated callus was represented as control. The mutagens stock solutions were filter sterilized using disposable microfilter (0.2μm Size). The 0.5g callus was soaked in each mutagen separately for ½ and 1hr, then blotted gently on sterilized filter papers and cultured on callus induction medium. All mutagen work was carried out under aseptic conditions in a laminar airflow cabinet. All cultures were maintained in temperature-controlled growth room at constant temperature (25±2ºC) with 16hr photoperiod under cool-white fluorescent light (1000 Lux irradiance) for 4 weeks. Fw of the untreated and treated calli were recorded on weekly basis to evaluate their growth

pattern. After 4 weeks, calli were harvested and subjected to FAs extraction for chromatographic analysis. The change in FA profiles of the produced mutant-H-calli was recorded for both mutagens.

Lipid extraction

The extraction procedure was performed using Soxhlet method according to A.O.A.C (2000). The harvested calli (control and treatments) were oven dried (Heraeus DVE oven) at 60°C for 2 days till constant weight. Certain weight (0.5g) of powdered calli was placed in thimble of the Soxhlet (Soxtherm Gerhadt laboratory instrument). Aliquot of 90ml petroleum ether was filled in round bottle flask and the whole Soxhlet was placed on a heating mantle to boil for 6hr. The condensing unit from extraction unit was removed and the sample(s) were allowed to cool down (contain all the lipids). The sample(s) were oven dried and placed in desiccator.

Separation of fatty acids

The separation of FAs was carried out according to the method described by Vogel (1975). 20% ethanolic-KOH was added to the sample(s) at room temperature for saponification. The sample(s) were acidified with 5N HCl to free their potassium salts and then extracted with petroleum ether at 40-60°C. The extract was washed three times with distilled water then dried using anhydrous Na_2SO_4 and filtered.

Methylation of fatty acids

The FAs of control, treatments and standards were dissolved in small amounts of anhydrous methanol and diazomethane ether until the gas effervescence stop. The mixture was left for 10min under nitrogen stream at room temperature to evaporate the ether. Few drops of chloroform (HPLC grade) were added to the methylated FAs. 10ml of each solution were used for FAs identification.

Identification of fatty acids

The FAs composition of the control and treatments was carried out with GLC according to the method described by Farag et al. (1986).

The samples were analyzed using PyeUnicam series 304 GLC with a dual flame ionization detector and a dual channel recorder. The separated methylated FAs were separated through a coiled glass column (1.5m x 4mm) packed with 100-120 mesh Diatomite-c and coated with 10%

683

CHANGES IN THE PROFILING OF FATTY ACIDSOF *GLYCINE MAX* L. (SOYBEAN) ...

polyethylene glycol adipate (PEGA). Temperature of injector and detector were 250 and 300°C, respectively. The column was programmed from 70 to 190°C (at rate of 8°C/min), then isothermally at 190°C for 25min with nitrogen at flow rate of 30ml/min. Methyl esters of FAs from C6:0 to C24:0 (Sigma Chemical Co., St Louis, MO, USA) were used as standards, (Table 1). The identification of FAs was accomplished by comparing the peaks of retention times with those of the corresponding standards. The quantity of individual compounds was determined by comparing the produced peak area with standard peak. The relation between the different concentrations of FAs and their peak area is represented in Fig. 4.

TABLE 1. Standard fatty acids used for Gas liquid chromatography (GLC) analysis.

| FAs		FAs	
Caproic acid	C6:0	Stearic acid	C18:0
Caprylic acid	C8:0	Oleic acid	C18:1
Pelargonic acid	C9:0	Linoleic acid	C18:2
Capric acid	C10:0	Linolenic acid	C18:3
Undecylic acid	C11:0	Nonadecylic acid	C19:0
Lauric acid	C12:0	Arachidic acid	C20:0
Tridecylic acid	C13:0	Heneicosylic acid	C21:0
Myristic acid	C14:0	Behenic acid	C22:0
Pentadecylic acid	C15:0	Tricosylic acid	C23:0
Palmitic acid	C16:0	Lignoceric acid	C24:0
Margaric acid	C17:0		

Statistical analyses

Means of triplicates and standard deviations were estimated for all recorded growth parameters. Growth dynamics values were statistically analyzed according to SPSS version 10 computer program (SPSS, 1999). To compare means, LSD test at levels of 5% and 1% was used (UPOV, 2007).

Results and Discussion

Seeds sterilization and germination

The sterilized seeds appeared clean and 1.5 folds larger than the dried ones (Fig 5). At the end of the experiment, the used sterilization technique showed no contamination, 20% non-viable seeds and 80% germination. Aseptically growing seedlings of 14 day-old were formed and used as a source of different explants for the next experiment.

In current investigation, disinfecting seeds with 2.625% NaOCl (concentration of 50% commercial Clorox) for few minutes produced 80% germination and 20% non-viability. Wide variety of chemicals and different techniques for seed sterilization were reported to eliminate microbes present on the seeds of soybean without destroying the viability of the seeds (Wilson, 1976; Caetano-Anolles et al., 1990; Phat et al., 2015). Clorox® and ethanol were the most common and safe sterilizing agents. In this context, Kim et al. (1990) and Bulbul et al. (2008), used those agents but with different concentrations and incubation times. They reported that NaOCl was effective as an external disinfecting and sterilizing agent against a broad range of microorganisms. However, Abdul-Baki (1974) found that this compound, in high concentrations, has strong oxidizing property which makes it highly reactive with amino acids, nucleic acids, amines, and amides causing the increase in dead seeds.
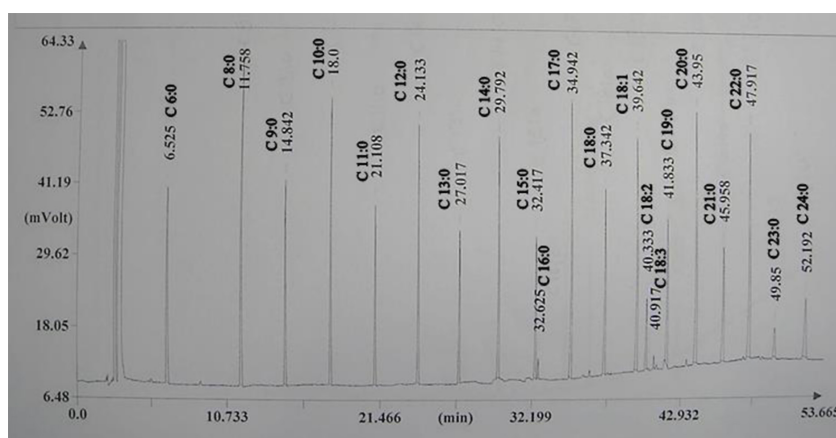
Fig. 4. Gas liquid chromatography (GLC) separation of methylated standard FAs.
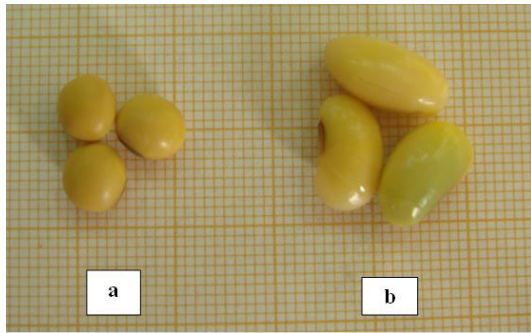
Fig. 5. Variation in the size between a) Non-sterilized and b) Sterilized seeds.

In present investigation, sterilized seeds were aseptically cultured on basal solid full-strength MS medium with vitamins and produced 80% germination. In this respect, Phat et al. (2015) reported that in soybean germination, solid medium induces efficient germination than liquid medium and MS with vitamins stimulate efficient germination compared to medium without vitamins.

Tissue culture

Establishment of callus cultures

Callus proliferation trials were performed by excised sterile H, E, C and L-explants from the 14 day-old aseptically growing seedlings (Fig. 3). Morphological variations were recorded at the end of the 1st and 2nd week of cultivation (Figs. 6, 7). Proliferation of calli at wounded edges of explants was initiated through the first week for most of the explants on certain media (Fig. 6). Dissimilar effects of 2,4-D and/or BA on calli proliferation of all explants were observed.

Concerning H-explants, variations in biomass of calli along the media were recorded. Media MS, M1, M5 and M7 showed no calli formation. M2 and M6 expressed slight induction of calli. Large and yellow calli were proliferated on both M3 (MS+ 3mg/L 2,4-D) and M4 (MS+ 0.5mg/L BA) (Fig. 6).

In case of C-explants, M3 and M4 media were effective in inducing yellow calli on all the cutted edges, which was more developed on M4 medium. M7 media showed small amount of calli; M2 along the explants cut edges while on M7 medium on the lower side of the C-explants. However, MS, M1, M5 and M6 media delayed the proliferation of calli (Fig. 6).

Concerning E-explants, all media showed the proliferation of calli except M6 and M7 media. M3 and M4 media were effective in inducing large yellow calli at both sides of the explants. However, only MS medium stimulated the formation of a root primordium.

Fig. 6. Morphogenic responses of different explants of soybean cultured *in vitro* for a week on MS medium supplemented with various combinations of 2,4-D and BA.

685

CHANGES IN THE PROFILING OF FATTY ACIDSOF *GLYCINE MAX* L. (SOYBEAN) ...
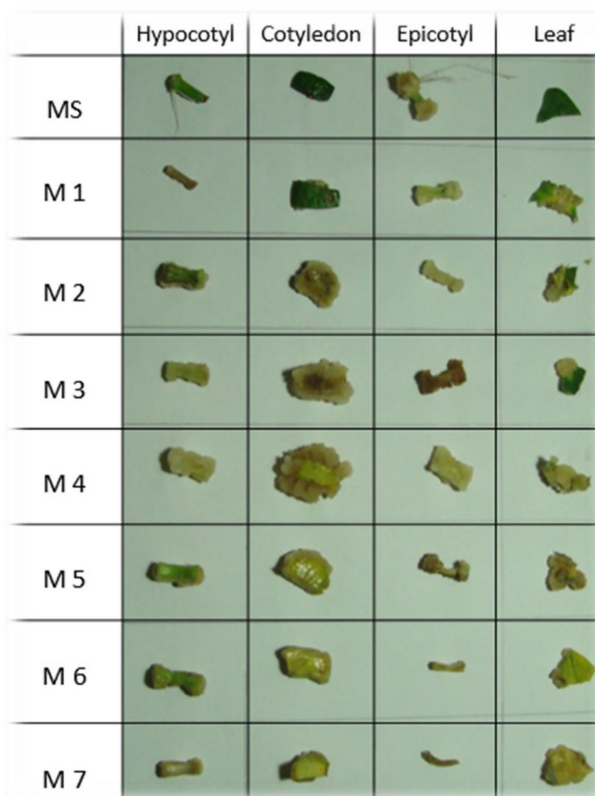
Fig. 7. Morphogenic responses of different explants of soybean cultured *in vitro* for 2 weeks on MS medium supplemented with various combinations of 2,4-D and BA.

L-explants cultured on M3, M4 and M5 attained medium amount of calli. M1, M2 and M7 medium showed the yellowing of the explants and no callus formation. M6 medium give very small amount of calli, while MS medium showed no responses (Fig. 6).

The dissimilar effects of 2,4-D and/or BA were still observed at the end of the second week (Fig. 7).

MS medium stimulated the formation of roots on both H- and E- explants; in addition, large yellowish-brown callus proliferated on both sides of the E-explants and no callus appeared on H-explants. Proliferated calli were amplified on the explants already having calli from the first week. M3 and M4 media showed the best responses for calli proliferation along all explants. Maximum proliferation of yellow calli appeared on H- and E- explants cultured on M4 medium. Brown color appears on C-calli on both M3 and M4 medium as well as on E-calli on M3 medium (Fig. 7).

Moderate amount of yellow calli appear on L-explants on both M3 and M4 media as well as H-explant on M3 medium. M1, M2, M6 and M7 medium showed the yellowing of the explants and started to form very little amount of callus. MS medium still showed no responses.

In this work, several trials were performed using different combinations of 2,4-D and BA to select the best medium for callus initiation and maintenance of different organs (H-, C-, E- and L-) of soybean *in vitro* plantlets. The results obtained showed that M4 medium gave the best results. Other media were excluded for either the presence of some morphogenic responses or the presence of calli with low biomass or brown color. Brown colors indicate the accumulation of phenolic and quinones compounds (Seitz et al., 1989), which may act as enzyme inhibitors (Harborne et al., 1975).

MS medium was widely used for callus initiation and maintenance of soybean by several investigators such as Joyner et al. (2010), Phat et al. (2015) and Abbasi et al. (2016). Additions

of myo-inositol and vitamins improved healthy callus growth (Narayanaswamy, 1994). Auxins and Cytokinins are plant growth regulators that interfered in various physiological growth processes (Rahdari & Sharifzadeh, 2012). Some reports mentioned the effect of using auxins and/or cytokinins for callus proliferations of different explants derived from *Glycine max* cultivars. Liu et al. (1997) proliferated well grown H-callus of soybean strain Tk5 when cultured on a medium containing both naphthalene acetic acid (NAA) and kinetin (Kn). Radhakrishnan & Ranjithakumari (2007) reported that the highest cell proliferation was obtained using half seed explant of the Indian soybean cv. CO3 in presence of 13.3μM benzyl amino purine (BAP) and 13.5μM 2,4-D. Joyner et al. (2010) reported that mature cotyledons of soybean cv. Pyramid produced high amounts of calli when cultured either in the media with 2,4-D or/and NAA. They also reported that all the used concentrations (3-21μM) of 2,4-D were able to proliferate calli. Phat et al. (2015) mentioned that cotyledonary node explants of Korean soybean cultivars Daepung and Nampung, induced callus when cultured in Gamborg B5 medium supplemented with 1mg L^{-1} BAP and 0.17mg L^{-1} gibberellic acid (GA3). They also emphasized that BAP had an important role in initiating and proliferating callus. However, Abbasi et al. (2016) showed that the best callus induction medium for shoot, root and L-explants of *G. max* L. cv. DPX was obtained by adding higher amounts of Kn than 2,4-D. Moreover, Devi et al. (2018) stimulated the highest callus biomass content of cotyledonary nodal leaves from indian soybean JS 335 on MS medium supplemented with 1.5mg/L 2,4-D and 0.1mg/L Kn. From the above available investigation, the divergence in the callus induction from the mentioned different soybean explants and cultivars in relation with various types and concentrations of growth regulators used may indicate the genotype specific responses.

Callus stocks

Based on the previously mentioned results, the M4 medium (MS+ 0.5mg/L BA) was the best medium among all tested media, giving healthy, yellow and rapidly growing callus proliferation for both H-and E-explants. Consequently, the initiated callus of both H- and E-explants was subcultured by transferring it every two weeks to new M4 medium (Fig. 8).

Growth dynamics of best callus

The growth characteristics of calli derived from H- and E-explants were cultured for 5 weeks on M4 medium. Calli growth which was expressed as Fw, Dw and GR were determined weekly along 5 weeks (Table 2). The data showed that the Fw and Dw insignificantly changed during the first week. Thereafter, high significant increment was recorded reaching maximum values at the 4th week.

Fig. 8. Callus stock of H-and E-explants after the first subculture on fresh M4 medium .

687

CHANGES IN THE PROFILING OF FATTY ACIDSOF *GLYCINE MAX* L. (SOYBEAN) ...

TABLE 2. Growth parameter of calli - derived from H- and E- explants of soybean cultured on M4 medium for 5 weeks.

Time (week)		Calli types					
		Hypocotyl			Epicotyl		
		FW	DW	GR	FW	DW	GR
0		0.40±0.03	0.04±0.001	–	0.40±0.02	0.04±0.002	–
1		0.61±0.01	0.05±0.01	0.02±0.001	0.52±0.03	0.05±0.003	0.01±0.01
2		0.94*±0.02	0.09**±0.004	0.03±0.001	0.77**±0.05	0.06±0.001	0.02±0.01
3		1.60**±0.06	0.14**±0.002	0.04±0.011	1.35**±0.1	0.08**±0.003	0.04±0.003
4		2.9**±0.06	0.25**±0.01	0.09±0.002	1.65**±0.05	0.11**±0.002	0.04±0.001
5		2.64**±0.02	0.23**±0.004	0.06±0.004	1.45**±0.02	0.09**±0.001	0.03±0.001
LSD	1%	0.52	0.04	–	0.25	0.01	–
	5%	0.38	0.03	–	0.18	0.01	–

- *= Significant at $P \leq 0.05$, **= Significant at $P \leq 0.01$
- Fw: Fresh weight, Dw: Dry weight, GR: Growth rate.

Concerning E-calli, the Fw did not show any significant changes during the first week and then high significant increases were recorded till the 4th week. Dw showed high significant increment till the 4th week. The Fw and Dw of both H- and E-explants decreased at the 5th week.

The results presented in Table 2 showed that the GR of calli derived from H- and E-explants cultured for 5 weeks on M4 medium continuously increased reaching optimum value at the 4th week, then decreased at the 5th week. At the 4th week, H-calli showed twice the GR of the E-calli.

It is interesting to mention here that during the four first weeks of cultivation, the calli of both explants were healthy and yellow. At the 5th week, all calli acquire yellowish-brown color.

Based on the previous results, the highest Fw and Dw as well as GR were recorded at the 4th week of subculture, so, it is the best harvesting time for both H- and E-calli. H-calli showed higher growth values than E-calli, so, they were selected as the source for Part 2 of the experiments.

Data of GR recorded in Table 2, illustrated the growth pattern of H-and E-calli cultures. After the first week of cultivation, little growth was recorded and moderate (for E-calli) to dramatic (for H-calli) increase in growth occurred during 2nd to 4th weeks.

At the 5th week, the growth slightly decreased.

The Fw and Dw as well as GR of H- and E-calli attained their maximum values at the 4th week of cultivation. H-calli gave better growth parameters than E-calli. In this connection, the growth parameters of both calli expressed a typical growth curves consisting of 3 phases: The lag phase was found at the 1st week in which no or only little growth was observed. Such effect was expected as the calli cultures adapted themselves on the new fresh subcultured medium (Kittipongpatana et al., 1998). In addition, Scragg & Allan (1993), considered this phase as an energy producer period. In the next phase, moderate to dramatic increase in growth occurred during 2nd to 4th weeks and was considered as log phase. At this stage of growth, the callus tissues enter a fast growing phase by synthesizing proteins, nucleic acid, phospholipids, as well as multiplication of cells, organelles and utilization of energy as ATP (Scragg & Allan, 1993; Kumar, 1999). After the log phase (5th weeks), the growth slightly decreased. This decline phase may be due to the depletion of nutrients in the medium as well as the reduction of the O_2 amounts inside the cells (Smith, 2000) or due the degradation of compounds over the synthesis processes and/ or due to the release of extracellular materials which accumulated in the medium and cannot be recovered by senescent calli (De & Roy, 1985).

Mutagenesis

Effect of different concentrations and soaking times in sodium azide (SA)

In this investigation, the Fw of the SA-treated H-calli showed a pattern similar to the control-calli but with lower values. The Fw decreased along the time of incubation as well as by increasing concentrations and soaking time of SA (Fig. 9).

In the available literature, no reports were recorded on the effect of SA on the calli growth of soybean but some papers focus on its impact on seed structures, germinations and growth of seedlings/plants (Abolude et al., 2012; Yu et al., 2012; Kumula & Sudjino, 2015). This decline in the calli weights with increasing SA concentrations was also observed in some leguminoseae as chickpea and mungbean (Khan et al. 2004, 2006), *Pisum sativum* and *Vicia faba* (Saad-Allah et al., 2014) as well as lentil (Sinha & Lai, 2007). Similar phenomenon was observed in calli of other plants such as *Daucus carota* L. (Nagananda et al., 2013) and *Mirabilis jalapa* (Al-Gawwad & Makka, 2009) and *Silybum marianum* (Abdel Rahman et al., 2015). They attributed this reduction to azide anions which cause the retardation of some biological and physiological processes through: (1) Mutation by interacting with enzyme activities and DNA in the cell (Mensah et al., 2006); (2) Inhibition of cytochrome oxidase, which in turn inhibit oxidative phosphorylation (Kleinhofs & Sander, 1975); (3) Alteration of the mitochondrial membrane potential (Zhang, 2000) and decrease

the cellular calmodulin level, Ca-binding-protein, for signal transduction and cell division, which inhibit the proton pump that blocks secretion and accumulation of cyclic adenosine monophosphate (cAMP) (Kleinhofs et al., 1978; Osborn & Weber, 1980; Dinauer et al., 1980). These effects together may hold ATP biosynthesis resulting in the decrease of accessibility of ATP which may slow the cell divisions and delay the GR of the treated-calli.

In this work, the results showed a simultaneous increment in both SFAs and UFAs, so, the balance between them were nearly unchanged (Table 3). Pentadecylic and Linolenic acids were increased constantly with increasing SA concentrations and soaking times, recoding their maximum values at 1.5mM SA. Linoleic acid was unaffected by SA-treatments. This increment in SFAs and UFAs may be due to the interaction performed by SA with enzymes involved in FAs metabolism present in mitochondria. In addition, the non-formation of oleic acid and the induction of high level of linolenic acid may be due to the activation of FAD enzymes involved in this conversion (Horwitz & Holt, 1971).

Effect of different concentrations and soaking times in ethidium bromide (EtBr)

In the present study, the Fw of the EtBr-treated calli increased very slowly by increasing concentrations and soaking times of EtBr (Fig. 10). The data also showed that the growth of the treated calli have lower values compared to control.
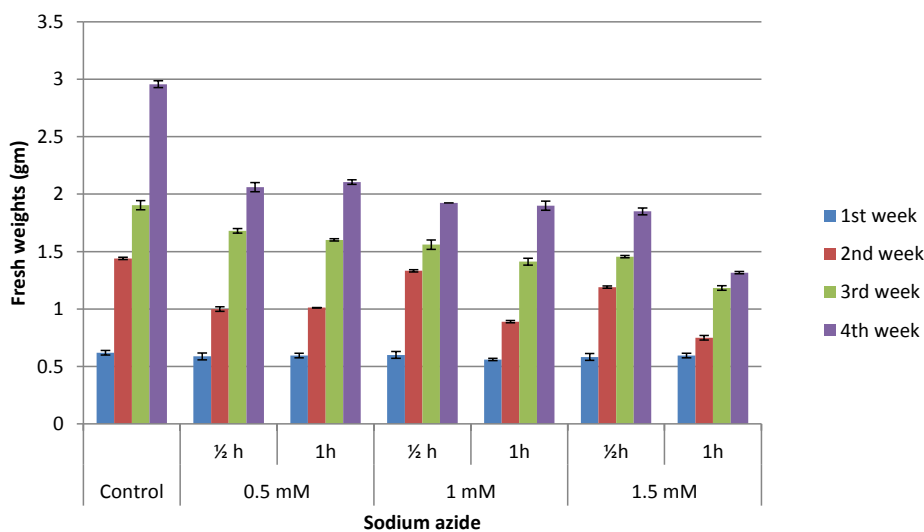

Fig. 9. Changes in Fw of H-calli of soybean after the treatments with different concentrations of SA at different time intervals along a month.

689

CHANGES IN THE PROFILING OF FATTY ACIDSOF *GLYCINE MAX* L. (SOYBEAN) ...

TABLE 3. GLC analysis of fatty acids of untreated and sodium azide-treated H-calli after 4 weeks of incubation.

FAs Name	Treatments		Control	0.5mM		1mM		1.5mM	
				½ h	1h	½ h	1h	½h	1h
SFAs	Pentadecylic acid	C15:0	2.95	3.35 (1.14)	4.46 (1.51)	5.17 (1.75)	10.99 (3.73)	11.58 (3.93)	12.05 (4.09)
	Stearic acid	C18:0	N.D.	N.D.	N.D..	N.D	N.D.	N.D.	N.D.
Total SFAs			**2.95**	**3.35**	**4.46**	**5.17**	**10.99**	**11.58**	**12.05**
UFAs	ω9 Oleic acid	C18:1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	ω6 Linoleic acid	C18:2	0.51	0.51	0.51	0.51	0.51	0.51	0.51
	ω3 Linolenic acid	C18:3	6.2	6.62 (1.07)	10.30 (1.66)	14.04 (2.27)	23.69 (3.82)	26.88 (4.34)	25.89 (4.18)
Total UFAs			**6.71**	**7.13**	**10.81**	**14.55**	**24.20**	**27.39**	**26.40**
Ratio SFAs: UFAs			**1:2.27**	**1:2.13**	**1:2.42**	**1:2.81**	**1:2.20**	**1:2.37**	**1:2.20**
Ratio ω3:ω6:ω9			**12.16:1:0**	**13:1:0**	**20.20:1:0**	**27.53:1:0**	**46.45:1:0**	**52.70:1:0**	**50.76:1:0**

- FAs: Fatty acids, SFAs: Saturated fatty acids, UFAs: Unsaturated fatty acids, N.D.: Not detected.
- Amounts of fatty acids are calculated as relative %.
- Data in bracket are the increment folds of control.
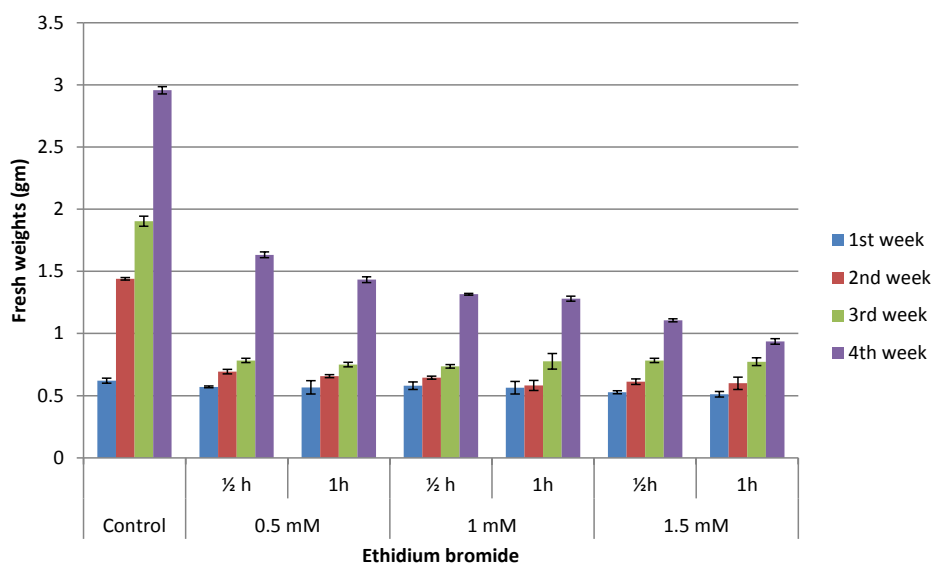
Fig. 10. Changes in Fw of H-calli of soybean after the treatments with different concentrations of EtBr at different time intervals along a month.

In this connection, it may be mentioned that no reports were recorded on the effect of EtBr on the calli growth of soybean. Few reports investigated the effect of EtBr on inflorescence-derived callus of *Sorghastrum nutans* (L.) (Stephens, 2009) and the germination of wheat, alfalfa and tomato (Amirijavid & Mohammadi, 2014). The marked decrease in growth of EtBr-treated-H-calli may be due to that EtBr is capable of (1) Disturbing the metabolism and protein synthesis of plants and so, the growth of the treated plants (Amirijavid & Mohammadi, 2014) and (2) Inhibiting the synthesis of mitochondrial macromolecules (Leblond-Larouche et al., 1979) by reducing mitochondrial DNA copy number (Henry et al, 1971; Hayakawa et al, 1998).

These effects together may affect the membranes constructions and hold ATP biosynthesis that slow the cell divisions and delay the GR of the treated-calli.

In this investigation, the results showed a complete change in the FAs profiling of the EtBr-treated-H-calli. Marked elevation of SFAs over UFAs in the EtBr-treated-H-calli was recorded exhibiting a switch in their balance as compared to the control (Table 4). EtBr is considered as an intercalating dye that inhibits mitochondrial DNA polymerase more effectively than nuclear DNA polymerase (Meyers & Simpson, 1969). It intercalates between two adenine-thymine base pairs hence, increasing the distance between adjacent base pairs causing mutation of DNA (Brown, 2002). The effect of EtBr on the mitochondria may cause the activation of some mitochondrial enzymes involved in the FAs pathway.

Conclusion

The present investigation revealed that the addition of 0.5mg/L BA to the MS medium produced the highest mass of healthy calli derived from H- and E-explants. Growth dynamics of those calli indicated that the harvesting time for both calli must be the 4th week and that H-calli had superiority over the E-calli in all the growth parameters.

The present study has also shown that both mutagens exhibited the same growth pattern as the control but with lower values. In addition, EtBr switched the balance between SFAs and UFAs in the treated H-calli by markedly elevated SFAs over UFAs. Moreover, the study showed that SA increased the ω-3 FAs over ω-6 ones.

We suggested that FAs produced from mutation treatments should be extracted and tested ethically on lab animals before human uses or consumptions.

Conflict of Interest: The authors declare no conflict of interest.

TABLE 4. GLC analysis of fatty acids of untreated and ethidium bromide-treated H-calli after 4 weeks of incubation.

FAs Names	Treatments		Control	0.5 mM		1 mM		1.5 mM	
				½ h	1h	½ h	1h	½h	1h
SFAs	Isocaproic acid	C6:0	N.D.	N.D.	0.12	0.19	0.19	0.20	0.23
	Caprylic acid	C8:0	N.D.	N.D.	0.07	0.13	0.10	0.19	0.18
	Pelargonic acid	C9:0	N.D.	N.D.	0.02	0.03	0.03	0.04	0.07
	Undecylic acid	C11:0	N.D.	0.36	0.38	0.75	1.33	1.14	1.48
	Tridecylic acid	C13:0	N.D.	1.59	2.17	4.21	4.59	6.27	8.83
	Myristic acid	C14:0	N.D.	1.04	1.72	3.31	4.04	4.92	6.29
	Pentadecylic acid	C15:0	2.95	1.33	1.36	1.98	2.46	3.65	5.01
	Margaric acid	C17:0	N.D.	4.72	6.16	6.71	9.72	9.98	10.89
	Stearic acid	C18:0	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.41
	Nondecylic acid	C19:0	N.D.	3.48	3.38	5.07	5.48	5.12	5.00
Total SFAs			**2.95**	**12.52**	**15.38**	**22.38**	**27.94**	**31.51**	**38.39**
UFAs	ω9 Oleic acid	C18:1	N.D.	1.85	2.34	2.54	2.58	3.63	4.09
	ω6 Linoleic acid	C18:2	0.51	N.D.	N.D.	N.D.	7.17	11.75	12.40
	ω3 Linolenic acid	C18:3	6.2	0.97	0.84	0.93	1.07	1.69	1.74
Total UFAs			**6.71**	**2.82**	**3.18**	**3.47**	**10.82**	**17.07**	**18.23**
Ratio SFAs: UFAs			**1:2.27**	**4.4:1**	**4.8:1**	**6.45:1**	**2.58:1**	**1.85:1**	**2.11:1**
Ratio ω3:ω6:ω9			**12.16:1:0**	**1:0:1.91**	**1:0:2.79**	**1:0:2.73**	**1:6.7 :2.4**	**1:6.95 :2.15**	**1: 7.13: 2.35**

- FAs: Fatty acids, SFAs: Saturated fatty acids, UFAs: Unsaturated fatty acids, N.D.: Not detected.
- Amounts of fatty acids are calculated as relative%

691

CHANGES IN THE PROFILING OF FATTY ACIDSOF *GLYCINE MAX* L. (SOYBEAN) ...

References

A.O.A.C. (2000) "*Official Methods of Analysis*" of the Association of Official Analytical Chemist, 14th ed., Washington, DC.

Abbasi, Z., Hooshyar, S., Bagherieh-Najjar, M.B. (2016) Improvement of callus production and shoot regeneration using various organs of soybean (*Glycine max* L. Merr) by response surface methodology. *In Vitro Cell Dev. Biol. Plant,* **52**, 537-545.

Abdel Rahman, R.A., Elfaramawy, A.M., Badr, A., Amer, M.A. (2015) Mutagenic effects of sodium azide and gamma rays on callus development and silymarin production in *Silybum marianum* (L.) Gaernt. *J. Pharm. Care Health Sys.* **2**(4), 108-109.

Abdul-Baki, A.A. (1974) Pitfalls in using sodium hypochlorite as a seed disinfectant in ^{14}C incorporation studies. *Plant Physiol.* **53**, 768-771.

Abolude, D.S., Adelanwa, M.A., Adelanwa, E.B., Adamu, S.M., Okafor, D.C. (2012) Morphological effects of sodium azide on soya bean (*Glycine max* (L) Merr). *Int. J. Scientific Res.* **2**(3), 115-123.

Al-Gawwad, A., Makka, A.H. (2009) Effect of chemical mutagens on Mirabilis jalapa. *M.Sc. Thesis*, Floriculture, Faculty of Agriculture, Alexandria University.

Amirijavid, Sh., Mohammadi, M. (2014) Toxicity of the ethidium bromide on germination of wheat, alfalfa and tomato. *Inter. Invention J. Agri. Soil Sci.* **2**(5), 69-74.

Brown, T.A. [Ed.] (2002) Mutation, Repair and Recombination, Chapter 14. In: "*Genomes*", 2nd ed. Oxford, Weley-Liss.

Bulbul, N., Sakurai, M., Matsushima, H., Kaneko, Y. (2008) Induction of ultrastructural specialization for ureide metabolism in non-nodule soybean tissues cultured *in vitro*. *Plant Sci.* **175**, 833-838

Caetano-Anolles, G., Favelukes, G., Bauer, W.D.

(1990) Optimization of surface sterilization for legume seed. *Crop Sci.* **30**, 708-712.

CarrollB, J., Mcneil, D.L., Gresshoff, P.M. (1986) Mutagenesis of soybean (*Glycine max* (L.) Merr.) and the isolation of non-nodulating mutants. *Plant Sci.* **47**, 109-114.

De, K.K., Roy, S.C. (1985) Morphogenetic investigation on pea under *in vitro* condition. *Bull. Torrey Bot. Club,* **112**, 363-367.

Devi, M.K.A., Kumar, S.S., Giridhar, P. (2018) High yield production of folates from soybean callus cultures in response to elicitors. *Biotech.* **8**, 80-89.

Dinauer, M.C., MacKay, S.A., Devreotes, P.N. (1980) Cyclic 3, 5-AMP relay in *Dictyostelium discoideum* III. The relationship of cAMP synthesis and secretion during the cAMP signaling response. *J. Cell Biol.* **86**, 537-545.

Dung, N.N., Szoke, E., Verzar-Petri, G. (1981) The growth dynamica of callus tissue of root and leaf origin in *Datura innoxia* Mill. *Acta Bot. Acad. Sci. Hung.* **27**, 325.

Farag, R.S., Hallabo, S.A.S., Hewedi, F.M., Basyony, A.E. (1986) Chemical evaluation of rape seed. *Fette-Seifen Anstrichmittel.* **88**(10), 391-397.

Gobinath, P., Pavadai, P. (2015) Morphology and yield parameters and biochemical analysis of soybean (*Glycine max* (L.) Mrr.) using gamma rays, EMS and des treatment. *Int. Letters Nat. Sci.* **35**, 50-58.

Hanafiah, D.S., Trikoesoemaningtyas, Y.S., Wirnas, D. (2010) Induced mutations by gamma ray irradiation to Argomulyo soybean (*Glycine max*) variety. *Biosci.* **2**(3), 121-125.

Harborne, J.B, Mabry, T.J., Mabry, H. (Eds.) (1975) "*The Flavonoids*", Part 2, Chapman and Hall Ltd. Academic Press, Inc., New York, San Francisco.

Hayakawa, T., Noda, M., Yasuda, K., Yorifuji, H., Taniguchi, S., Miwa, I., Sakura, H., Terauchi, Y., Hayashi, J., Sharp, G.W., Kanazawa, Y., Akanuma, Y., Yazaki, Y., Kadowaki, T. (1998) Ethidium bromideinduced inhibition of mitochondrial gene transcription suppresses glucose-stimulated insulin release in the mouse pancreatic beta-cell line betaHC9. *J. Biol. Chem.* **273**(32), 20300-20307.

Henry, B., Holt, H.E., Holt, H.C. (1971) Specific inhibition by ethidium bromide of mitochondrial DNA synthesis in *Physarum polycephalum*. *J. Cell Biol.* **49**(2), 546-553.

Horwitz, H.B., Holt, C.E. (1971) Specific inhibition by ethidium bromide of mitochondrial DNA synthesis in *Physarum polycephalum*. *J. Cell Biol.* **49**, 546-553.

Joyner, E.Y., Boykin, L.S., Lodhi, M.A. (2010) Callus induction and organogenesis in soybean [*Glycine max* (L.) Merr.] cv. Pyramid from mature cotyledons and embryos. *Open Plant Sci. J.* **4**(1), 18-21.

Karthika, R., Lakshmi, B.S. (2006) Mutagenic effectiveness and efficiency in soybean. *Plant Archives*, **6**(1), 277-279.

Khan, M.H., Tyagi, S.D. (2013) A review on induced mutagenesis in soybean. *J. Cereals and Oilseeds,* **4**(2), 19-25.

Khan, M.H., Tyagi, S.D. (2017) Chlorophyll and morphological mutation induction in soybean (*Glycine max* (L.) Merrill.) through modified single seed bulk method Academia. *J. Agri. Res.* **5**(9), 218-223.

Khan, S., Wani, M.R., Bhat, M., Parveen, K. (2004) Induction of morphological mutants in chickpea. *Int. Chickpea Pigeonpea Newsletter*, **11**, 6-7.

Khan, S., Wani, M.R., Parveen, K. (2006) Quantitative variability in mungbean induced by chemical mutagens. *Legume Res.* **29**, 143-145.

Kim, J., LaMotte, C.E., Hack, E. (1990) Plant regeneration *in vitro* from primary leaf nodes of soybean (*Glycine max*) seedlings. *J. Plant Physiol.* **136**, 664-669.

Kittipongpatana, N., Hock, R.S., Porter, J.R. (1998) Production of solasodine by hairy root, callus and cell suspension cultures of *Solanum aviculare* Forst. *Plant Cell Tiss. Organ Cult.* **52**, 133-143.

Kleinhofs, A., Sander, C. (1975) Azide mutagenesis in barley. *Proceedings of the 3rd International Barley Genetics Symposium*, July 7-12, Garching, Germany, pp. 113-122.

Kleinhofs, A., Owais, W., Nilan, R. (1978) Azide. *Mutat Res.* **55**(3), 165-195.

Kotchoni, S.O., Noumavo, P.A., Adjanohoun, A., Russo, D.P., Dell'Angelo, J., Gachomo, E.W., Baba-Moussa, L. (2012) A simple and efficient seed-based approach to induce callus production from B73 maize genotype. *Am. J. Mol. Biol.* **2**, 380-385.

Kumar, U. [Ed.] (1999) "*Studies in Biotechnology*" series 4: Methods in Plant Tissue Culture, Agro. Botanica, India.

Kumula, D., Sudjino (2015) Growth and development of soybean (*Glycine max* l.) treated with sodium azide. *The 3rd International Conference on Biological Science* (ICBS-2013), Vol 2, 632p. https://knepublishing.com/index.php/Kne-Life/article/view/234/1427.

Leblond-Larouche, L., Morais, R., Zollinger, M. (1979) Studies of the effect of chloramphenicol, ethidium bromide and camptothecin on the reproduction of *Rous sarcoma* virus in infected chick embryo cells. *J. Gen. Virol.* **44**, 323-331.

Lee, J.D., Bilyeu, K.D., Shannon, J.G. (2007) Genetics and breeding for modified fatty acid profile in soybean seed oil-review. *J. Crop Sci. Biotech.* **10** (4), 201-210.

Liu, Z.H., Wang, W.C., Yan, S.Y. (1997) Effect of hormone treatment on callus formation and endogenous indole-acetic acid and polyamine contents of soybean hypocotyl cultivated *in vitro*. *Bot. Bull. Acad Sin.* **38**, 171-176.

Masuda, T., Goldsmith, P.D. (2009) World soybean production: Area harvested, yield, and long-term projections. *Int. Food Agribus. Man. Rev.* **12**(4), 143-162.

Mensah, J.K., Obadoni, B.O., Akomeah, P.A., Ikhajiagbe, B., Ajibolu, J. (2006) The effects of sodium azide and colchicine treatments on morphological and yield traits of sesame seed (*Sesame indicum* L.). *Afr. J. Biotechnol.* **6**(5), 534-538.

Meyers, R.R., Simpson, M.V. (1969) DNA biosynthesis in mitochondria: Differential inhibition of mitochondrial and nuclear DNA polymerases by the mutagenic dyes ethidium bromide and acriflavin.

693

CHANGES IN THE PROFILING OF FATTY ACIDSOF *GLYCINE MAX* L. (SOYBEAN) ...

Biochem. Biophys. Res. Commun. **34**(2), 238-244.

Murashige, T., Skoog, F. (1962) A revised medium for rapid growth and biossays with tobaco tissue cultures. *Physiologia Plantarum*, **15**(3), 473-479.

Nagananda, G.S., Rajath, S., Agarwal, D., Mathew, K.R., Rajan, S.S. (2013) Induced mutation in callus Cell lines of *Daucus carota* L. *Asian J. Plant Sci.* **12**(1), 46-50.

Napier, J.A., Michaelson, L.V. (2001) Towards the production of pharmaceutical fatty acids in transgenic plants. *J. Sci. Food Agri.* **81**, 883-888.

Narayanaswamy, S. [Ed.] (1994) *Plant Cell and Tissue Culture*, pp.651, Tate McGrew, Hill Publishing Company Limited, New Delhi.

Nikolić, N., Stanković, M., Todorović, Z., Lazić, M., Nikolić, G. (2008) HPLC analysis of whole soybean (*Glycine max* L.) seed oil. *Acta Agriculturae Serbica*, **25**, 3-8.

Olguin, M.C., Hisano, N., D'Ottavio, E.A., Zingale, M.I., Revelant, G.C., Calderari, S.A. (2003) Nutritional and antinutritional aspect of an Argentinean soy flour assessed on weanling rats. *J. Food Comp. Anal.* **16**, 441-448.

Osborn, M., Weber, K. (1980) Damaged cellular functions by trifluoperazine, a calmodulin specific drug. *Exp. Cell Res.* **130**, 484-489.

Patil, A., Taware, S.P., Oak, M.D., Tamhankar, S.A., Rao, V.S. (2007) Improvement of oil quality in soybean [*Glycine max* (L.) Merrill] by mutation breeding. *J. Am. Oil Chem. Soc.* **84**, 1117-1124.

Patil, G.P., Wakode, M.M. (2011) Effect of physical and chemical mutagens on soybean. *Curr. Bot.* **2**(4), 12-14.

Phat, P., Rehman, S.Ur., Jung, H-I., Ju, H-J. (2015) Optimization of soybean (*Glycine max* L.) regeneration for Korean cultivars. *Pak. J. Bot.* **47**(6), 2379-2385.

Radhakrishnan, R., Ranjithakumari, B.D. (2007) Callus induction and plant regeneration of Indian soybean [*Glycine max* (L.) Merr. cv. CO3] via half seed explants culture. *J. Agri. Technol.* **3**, 287-297.

Rahdari, P., Sharifzadeh, V. (2012) Effects of auxin and cytokininon morphological and physiological factors in soybean (*Glycine max* L). *Life Sci. J.* **4**, 5796-5807.

Rahman, S.M., Takagi, Y., Kubota, K., Miyamoto, K., Kawakita, T. (1994) High oleic acid mutant in soybean induced by X-ray irradiation. *Biosc. Biotechnol. Biochem.* **58**(6), 1070-1072.

Rahman, S.M., Takagi, Y., Kubota, K., Miyamoto, K., Kawakita, T. (1995) High stearic acid soybean mutant induced by X--ray irradiation. *Biosci. Biotech. Biochem.* **59**(5), 922-923.

Saad-Allah, K.M., Hammouda, M., Kasim, W.A. (2014) Effect of sodium azide on growth criteria, some metabolites, mitotic index and chromosomal abnormalities in *Pisum sativum* and *Vicia faba*. *Int. J. Agro. Agri. Res.* **4**, 129-147.

Sagel, Z., Tutluer, M.İ., Peskircioglu, H., Kantoglu, Y., Kunter, B. (2017) Determination of effect of chemical mutagen EMS on TAEK A-3 and TAEK C-10 mutant soybean varieties in M1 generation. *Ekin. J. Crop Breed Gen.* **3**(1), 19-24.

Sahena, F., Zaidul, I.S.M., Jinap, S., Karim, A.A., Abbas, K.A., Norulaini, N.A.N., Omar, A.K.M. (2009) Application of supercritical CO_2 in lipid extraction – A review. *J. Food Engin.* **95**, 240-253.

Scragg, A.H., Allan, E.J. (1993) *Picrasma quassioides* Bennet (Japanese quassia tree): *In vitro* culture and production ofquassin. In: "*Biotechnology in Agriculture and Foresty: Medicinal and Aromatic Plants*", Bajaj, Y.P.S. (Ed.), pp. 249-268. Berlin: Springer-Verlag.

Seitz, H.U., Eilert, U., DeLuca, V., Kurz, W.G.W. (1989) Elicitor-mediated induction of phenylalanine ammonia lyase and tryptophan decarboxylase: Accumulation of phenols and indole alkaloids in cell suspension cultures of *Catharanthus roseus*. *Plant Cell Tiss. Org. Cult.* **19**(1), 71-78.

Shanab, S.M.M., Hafez, R.M., Fouad, A.S. (2018) A review on algae and plants as potential source of arachidonic acid. *J. Adv. Res.* **11**, 3-13

Sinha, A., Lai, J.P. (2007) Effect of mutagens on M parameters and qualitative changes induced in M generation in lentil. *Legume Res. Int. J.* **30**, 180-185.

Slater, A., Scott, N.W., Fowler, M.R. (2008) "*Plant Biotechnology: The Genetic Manipulation of Plants*", 2nd ed. Oxford University Press, Oxford.

Smith, R.M. (2000) "*Plant Tissue Culture: Techniques and Experiments*", San Diego: Academic, 231p.

Soares, B.M.C., Gamarra, F.M.C., Paviani, L.C., Goncalves, L.A.G., Cabral, F.A. (2007) Solubility of triacylglycerols in supercritical carbon dioxide. *J. Supercrit Fluids,* **43**, 25-31.

Soystat (2010) http://soystats.com/2010/page_30.htm [Retrieved on August 5, 2010].

SPSS (1999) SPSS for Windows, version 10.0. SPSS Inc., Chicago, 233S, Wacker Drive, Chicago, Illinois, USA.

Srivastava, D.K., Gupta, V.K., Sharma, D.R. (1995) *In vitro* selection and characterization of water stress tolerant callus cultures of tomato (*Lycopersicon esculentum* L. Mill cv. Ktl.). *Indian J. Plant Physiol.* **38**, 99-104.

Stephens, L.C. (2009) Ethidium bromide-induced mutations from inflorescence cultures of Indiangrass. *Hort. Sci.* **44**(5), 1215-1218.

UPOV (2007) Least significant difference (LSD), technical working party on automation and computer programs, International Union for the Protection of new Varieties of Plants, Sibiu, Romania. https://www.upov.int/edocs/mdocs/upov/en/twc/25/twc_25_09_rev.pdf

Vogel, A.J. (1975) "*A Text Book of Practical Organic Chemistry*", 3rd ed., English Language Book Society and Longman Group Ltd., London.

Wilcox, J.R., Cavins, J.F., Nielsen, N.C. (1984) Genetic alteration of soybean oil composition by a chemical mutagen. *J. Am. Oil Chem. Soc.* **61**(1), 97-100.

Wilson, R.F., Rinne, R.W., Brim, E.A. (1976) Alteration of soybean oil com position by plant breeding. *J. Am. Oil Chem. Soc.* **53**, 595-597.

Yu, J., Han, S., Shi, G., Yu, D. (2012) Characterization of a novel *curled-cotyledons* mutant in soybean [*Glycine max* (L.) Merr.]. *Afr. J. Biotechnol.* **11**(83), 14889-14898.

Zhang, B.H. (2000) Regulation of plant growth regulators on cotton somatic embryogenesis and plant regeneration. *Biochem.* **39**, 15-67.

التغيرات في نمط صوره الأحماض الدهنية في كالوس .Glycine max L (فول الصويا) بعد المعامله بالمطفرات

رحاب محمود حافظ⁽¹⁾، عبد الله احمد ياسين محمد⁽²⁾، عبد الرحمن محمد عبد النبي⁽²⁾، علي السيد علي طلبه⁽²⁾، اسلام يوسف محمد خليفه⁽²⁾، حامد محمود حامد⁽²⁾، محمد محسن خالد عبد الله⁽²⁾، محمد مجدي فتحي احمد⁽²⁾، محمد شعبان هيكل⁽²⁾، داليا حمدي أحمد علي⁽²⁾

⁽¹⁾قسم النبات و الميكروبيولوجي - كليه العلوم - جامعه القاهرة - جيزه 12613، مصر،⁽²⁾قسم الكيمياء - كليه العلوم - جامعه القاهرة - جيزه 12613 - مصر.

كان الهدف من الدراسة الحالية هو إنتاج كالوس صحي لين من صنف فول الصويا جيزة 111 ودراسة تأثير الصوديوم أزيد (SA) وبروميد الإيثيديوم (EtBr) المطفرة على نمط صوره الأحماض الدهنية (FAs) . وقد تم تعقيم البذور باستخدام الكلوروكس والإيثانول، ثم تم استزراعها على وسط موراشيج وسكوج (MS) لإنتاج نبتات معمليا عمرها 14 يومًا. تم اختبار تكوين الكالوس من عزلات الساق الجنينيه العلويه والفلقه و السويقه الجنينيه السفلي والورقه باستخدام تركيزات مختلفه من هرمون 2,4-D مع/او BA. وقد وجد أن البيئه M4 التي تحتوي على MS بالإضافة إلى 0.5 ملليجرام/لتر BA تكون أعلى مستوى لإنتاج الكالوس. كانت أعلى العزلات إنتاجا للكالوس هي الساق الجنينيه العلويه والسويقه الجنينيه السفلي. تم تقدير ديناميكيات النمو لكالوس الساق الجنينيه العلويه و السويقه الجنينيه السفلي. واتضحت أن أفضل وقت لحصاد الكالوس لهذه العزلات هو الأسبوع الرابع. وكان كالوس الساق الجنينيه العلويه هي الأكثر سرعة في النمو. تم معامله كالوس الساق الجنينيه العلويه بتركيزات 0.5، 1.0 و1.5 ملم من كل مطفرعلى حده مع اثنين من فترات النقع (½ ساعه و1ساعه) ثم استزراعها على البيئه. بعد شهر، تم إجراء تحليل لمعرف نمطها و صوره الأحماض الدهنيه في الكالوس المعامل بالمطفرات و الغير معامل باستخدام جهاز الكروماتوجرافي الغاز-السائل (GLC). وأظهرت النتائج أن نمط الوزن الطازج للكالوس بعد المعامله بالمطفرات متشابهين مع مثيلاتها للكالوس الغير معامل ولكن بقيم أقل. عند تحليل نمط الأحماض الدهنيه المختلفه اتضح زياده الأنوع المشبعة (SFAs) والغير مشبعه (UFAs) بزيادة تركيزات الصوديوم أزيد مع أوقات النقع. تم أيضا تسجيل ارتفاع ملحوظ لأنواع الأحماض الدهنيه المشبعه على مثيلاتها غير المشبعه عند معامله الكالوس ببروميد الإيثيديوم، مما يدل على تحول التوازن الأصلي بينهما عن طريق زيادة التركيزات وأوقات النقع.