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EVALUATION OF ADVANCED GENERATIONS OF EARLY FLOWERING AND HEAVY BRANCHING/HIGH YIELDING MUTANTS OF EGYPTIAN LUPINE
II-CHEMICAL FEATURES OF MUTANTS

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ABSTRACT

Four mutants (two early flowering and two heavy branching/high yield) obtained from Egyptian lupine cv.Giza 2 after treating seeds with either ethylmethane sulphonate (EMS) or sodium azide (SA), Hassan (1991 and 1998) in a mutation breeding programme conducted at the Agricultural Experiments and Researches Station, Faculty of Agriculture, University of Cairo, Giza, Egypt since 1987. In M7-generation, these mutants were evaluated for alkaloid and fatty acid composition of the oil.

GLC analysis proved the presence of six alkaloids in lupine seeds under investigation. Lupanine represents the major component (56.32-63.34%) in all investigated seeds followed by 13&-hydroxylupanine (12.40-17.32%) then by multiflorine (8.64-13.74). Whereas, albine (3.38-9.02), angustifoline (4.09-6.75%) and 11,12-Seco-12,13-didehydromultiflorine (2.59-5.93%) represent the minor components. It is noted that the increase in total alkaloid content, which was observed in seeds of the heavy branching/high yield (G2 EMS HB/HY6), early flowering (G2 EMS EF1) and early flowering (G2 SA EF2) mutants, was reflected in increased concentration of individual components. Likewise, the reduction in total alkaloid content, which was observed in the heavy branching/high yield (G2 SA HB/HY3), was reflected in reduced concentration of individual components. On the other hand, GLC analysis proved the presence of seven fatty acids beside two unknowns in lupine seeds under investigation. The higher relative concentration was for fatty acid C18:1 (39.6-47.9%) in all investigated seeds, followed by C18:2 (21.4-24.8%), while C16:0 and C18:3 reached (16.6-18.2%) and (8.3-12.4%); respectively. The other fatty acids, C18:0, C20:0, C22:0, unknown1 and unknown 2 were present in lesser amounts in all investigated seeds ranged from traces to 2.2%. It is noted that the increase in total fatty acids content, which was observed in seeds of the heavy branching/high yield and early flowering mutants, reflected in increased concentration of individual components.

Keywords: Evaluation, early flowering, heavy branching/high yielding, mutants, Egyptian lupine, chemical features

INTRODUCTION

The real value of lupine for foods and feeds is referred to its high content of protein which is considered as a good source of lysine and generally poor in the sulphur containing amino acids (Digna *et al.*, 1980). Other interest is the relatively high content of oil in some lupine varieties and certain vitamins which are not found in other legumes or soyabeans. In fact, as a human food, the absence of protease inhibitors and haemagglutinins gives lupines a slight quality advantage over soya.In general, lupines demonstrate lower antitryptic, allergenic and flatulence factors (oligosaccharides) than other pulses, including soya (Belteky and Kovacs, 1984).

Lupine alkaloids have a number of pharmacological activities, such as antiviral, antibacterial, antifungal, mollusc deterrence, natural insecticides, against herbivores and competing weeds (Wink,1992 and 1993). Many reports have shown that Egyptian lupine could be used in many different aspects. Seeds are vermifuge. The liquid left after soaking the bitter seeds in water is used as a parasiticide, emollient of the skin for scurf, tinea and itch. Cataplasm of seeds is emollient, resolvent. Powdered dry seeds are taken against diabetes (Boulos, 1983).

Induced mutagenesis is a useful technique when variability cannot be found in the species and for characters controlled by a few genes. In previous mutation studies in *Lupinus*, in which seeds were treated with chemical and physical mutagens, some mutants with low or high alkaloid content, high protein and oil content and many other economical characteristics were selected by many workers, Pakendorf (1974), Fartushnyak (1974), Von Baer and Gross (1977), Sanaev (1979), Jayasekera (1981), Harrison and Williams (1982), Williams *et al.* (1984), Klochko *et al.* (1992), Gataulina (1994). In addition, the induction of genetic variability by mutagenesis has made possible the identification and isolation of mutants with altered fatty acid composition in the seed lipids of commercial oilseed crops. These mutant lines in soybean (*Glycine max* (L.) Merr.) include a high C18:0 (Graef *et al.*,1985), a high oleic acid (C18:1) or low linolenic acid (C18:3) content (Wilcox *et al.*, 1984), a high or low palmitic acid (C16:0) conent (Wilcox and Cavins,1990), a high palmitic acid (16:0) content (Fehr *et al.*, 1991) and a high linolenic acid (C18:3) content (Rahman *et al.*,1994) and in sunflower high stearic acid (C18:0) or palmitic acid (C16:0) mutants (Osorio et al.,1995).

In the first paper of this study, the author (2002) evaluated five true breeding M5-mutant families belong to two different mutant types (two early flowering and three heavy branching/high yield mutants). These mutants were raised to study their behaviour and performance, compared to the parental lupine cv. Giza 2, in M6 and M7 generations. Moreover, the evaluation of selected mutants includes anatomical studies.

The present work (the second part of this study) was conducted to complement such mutation breeding programme to evaluate four true breeding M5-mutant families belong to two different mutant types (two early flowering and two heavy branching/high yield mutants) derived from Egyptian lupine cv. Giza 2 after treating seeds with either EMS or SA (Hassan, 1991 and 1998). The evaluation of selected mutants includes the investigations on chemical composition of the seeds especially their lipids and alkaloid contents.

MATERIALS AND METHODS

The mutants used for this investigation were derived from Egyptian lupine cv. Giza 2 after treating seeds with either ethylmethane sulphonate (EMS) or sodium azide (SA), Hassan (1991and 1998) in a mutation breeding programme conducted at the Agricultural Experiments and Researches Station, Faculty of Agriculture, University of Cairo, Giza, Egypt since 1987.

B) Extraction of fatty acids compostion:

The fatty acids were separated and fractionated according to the method of Kolattukudy and Agrawal (1974). One gram of the dried seed sample was continuously extracted with a 1:1 mixture of chloroform and methanol (60 ^{0}C) for 24 hours in a Soxhlet apparatus. The solvent was evaporated under an atmosphere of nitrogen and the slurry of lipids was homogenized in 25ml. bortrifluorid (BF3) 15% in methanol after addition of 2mg heptadecanoic acid (C17:0) of it to each particular fraction of extracted lipids was used as an internal standard because this substance is normally absent from biological material and the mixture was left standing at room temperature overnight under an atmosphere of nitrogen. The homogenate was then transferred to a separator funnel with approximately 25ml. of chloroform and 50ml. distilled water. The solution was extracted with four sequential amounts of chloroform. The combined chloroform phases were saponified with 40ml. 1N KOH in a separation funnel. Thereafter, the combined extracts were transferred again to a separation funnel and acidified by 40ml. 3N HCL. The chloroform of the remaining lipid fraction was evaporated to dryness under reduced pressure. For GLC analysis the dried residue was redissolved in a known volume (5ml.) of chloroform sealed under a nitrogen atmosphere in a dark bottle and stored at –20 ^{0}C in a refrigerator. A standard solution cocktail consisting of a mixture of authentic samples of fatty acid methyl esters diluted in a known proportion of chloroform was run under the same experimental condition prior to running the samples. The retention times of peaks, obtained from the separation of the unknown samples of methyl esters, were compared with those of the known standards for identification purposes.

GLC analysis:

The fractionation of fatty acid methyl esters was conducted by using PYE UNICAM series 204 gas chromatography equipped with a fused silica capillary columns (glass column 25mX0.25mm) and flame ionization detectors. GLC conditions: detector and injector temperatures were set at 250 ^{0}C and 200 ^{0}C; respectively; carrier gas: nitrogen (flow rate 20 ml./min.). The oven temperature programme starting with 75 ^{0}C (0.0 min. isothermal, 12 ^{0}C/min. to 193 ^{0}C, then isothermally at 193 ^{0}C for 44 min.).

RESULTS AND DISCUSSION

1-GLC-analysis of alkaloids:

Seed alkaloids were analyzed by capillary GLC and GLC-MS. The obtained results proved the presence of six alkaloids from *Lupinus termis* Forssk.cv. Giza 2 according to their retention times(Rt) comparing with those of authentic samples and comparing their Kovats retention indexes (RI) and their characteristic mass spectra (MS) with literature data (Wink *et al.*, 1980; Wink *et al.*, 1983; Wink and Witte (1984); Meiβner and Wink,1992; Wink,1992; Bäumel *et al.*,1993; Planchuelo-Ravelo and Wink,1993; Bäumel *et al.*,1995 and Wink *et al.*,1995), Figures (1).The obtained six alkaloids were identified as Albine: RI = 1900; MS (in bractes: relative abundance of ions): 191(100), 110(61), 232(23) 122(22), 82(18), 54(16); Angustifoline: RI =2083; MS: 193(100), 112(67), 55(20),

The mutant types are: two early flowering mutants (G2 EMS EF1 and G2 SA EF2) derived from 0.10% EMS, 2 X 10^{-3} M SA; respectively and two heavy branching/high yield mutants (G2 EMS HB/HY3 and G2 EMS HB/HY6) derived from 0.25% EMS, 2X10^{-3}M SA; respectively. Details of the cultivar characteristics, concentrations and method of the chemical mutagens application and handling the materials during the successive M1 to M5 generations are stated by Hassan (1991 and 1998).

The field work was carried out at the Agricultural Experiments and Researches Station, Faculty of Agriculture, University of Cairo, Giza, Egypt, during the two growing seasons of 1998/99 and 1999/2000, M6 and M7-generations; respectively. Seeds of each M7-selected family within each mutant type as well as from the control (normal lupine cv. Giza 2) were used to analyze the alkaloids and fatty acids composition.

A) Extraction of Alkaloids:

The alkaloids samples were separated and fractionated according to the method of Wink *et al.* (1995). A sample of 500mg (powder of seeds) was homogenized in 15 ml. 1M HCl in a Waring blender and left standing at room temperature for 24 hours. Then the homogenate was filtered through nylon gauze (100µm mesh). The filtrate was made alkaline by adding 6M NaOH and was applied onto a standard Extrelut columns(Merk, Darmstadt).The alkaloids were eluted three times with 100 ml. dichloromethane (CH2Cl) and the extracts were evaporated to dryness.

The alkaloid extract was dissolved in 3ml.methanol using 1ml. for each 0.17g of original plant material. 1µl of this extract solution was injected into the column. A standard solution cocktail consisting of a mixture of authentic samples of alkaloids diluted in a known proportion of methanol was run under the same experimental conditions prior to running the samples. Peak identification was performed by comparing the retention time (Rt) of each compound with those of standard materials.

Gas-Liquid Chromatography(GLC):

A Carlo Erba MFC 500 gas chromatograph equipped with FID, spectra physics integrator, OVI fused silica capillary columns (15mX0.25 mm); carrier gas: He (flow rate 2 ml./min.); split ratio 1:20; detector temperature 300 ^{0}C; injector temperature 250 ^{0}C and oven temperature programme starting with 150 ^{0}C(3 min. isothermal, 10 ^{0}C/min. to 250 ^{0}C, 15 ^{0}C/min. to 300 ^{0}C, 10 min. isothermal).

GLC-MS analysis:

A Carlo Erba HRGC 4160 gas chromatograph equipped with a fused silica capillary columns OVI (30m) coupled to a quadrupole mass spectrometer Finnigan MAT 4500 was employed. EI-mass spectra were recorded at 40 eV. For GLC-MS the following conditions were used: injector temperature 250 ^{0}C; split ratio 1:20; carrier gas: He (flow rate 1 ml./min.); temperature programme: 150 ^{0}C (3 min. isothermal 10 ^{0}C/min. to 250 ^{0}C, 15 ^{0}C/min. to 300 ^{0}C, 10 min. isothermal). The identification of the compounds was based on a comparison of retention indexes and mass spectra with those of authentic samples and with literature data.

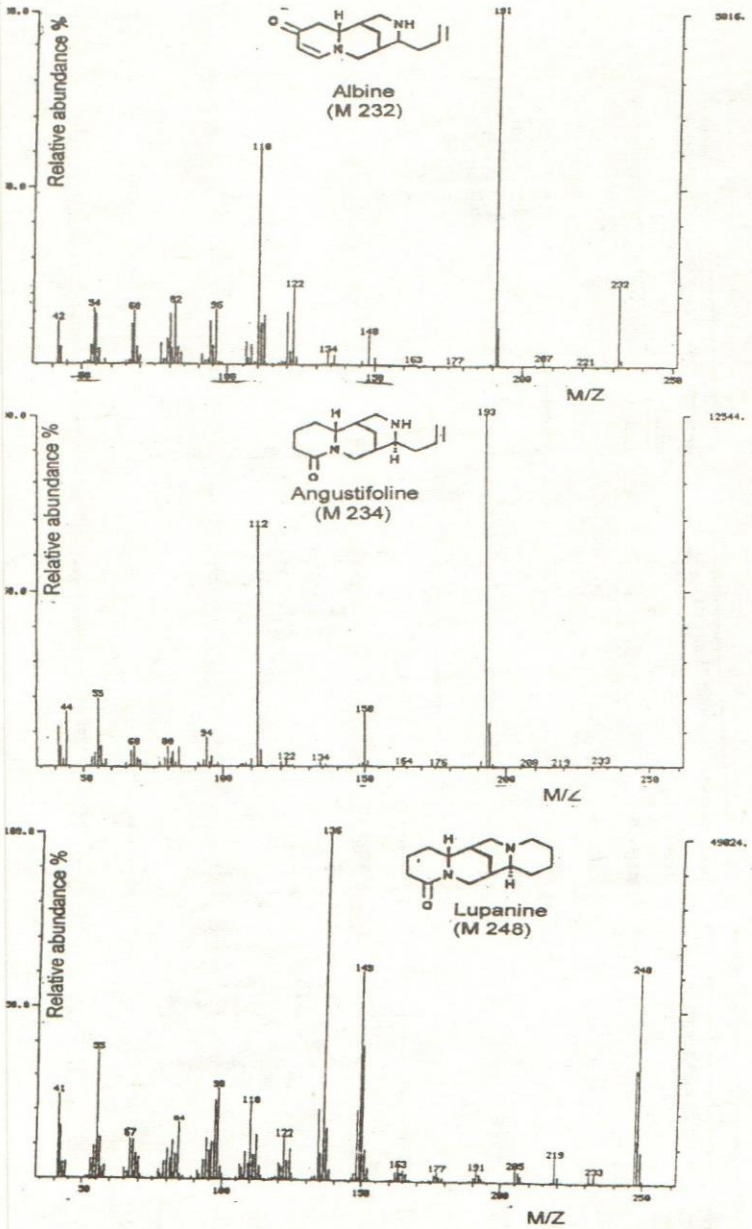

Figure (1): Mass spectra of six alkaloids (Albine, Angustifoline, Lupanine, 11,12– Seco - 12,13 -didehydromultiflorine, Multiflorine and 13&-hydroxylupanine) identified in Lupinus termis Forssk. Cv. Giza 2.

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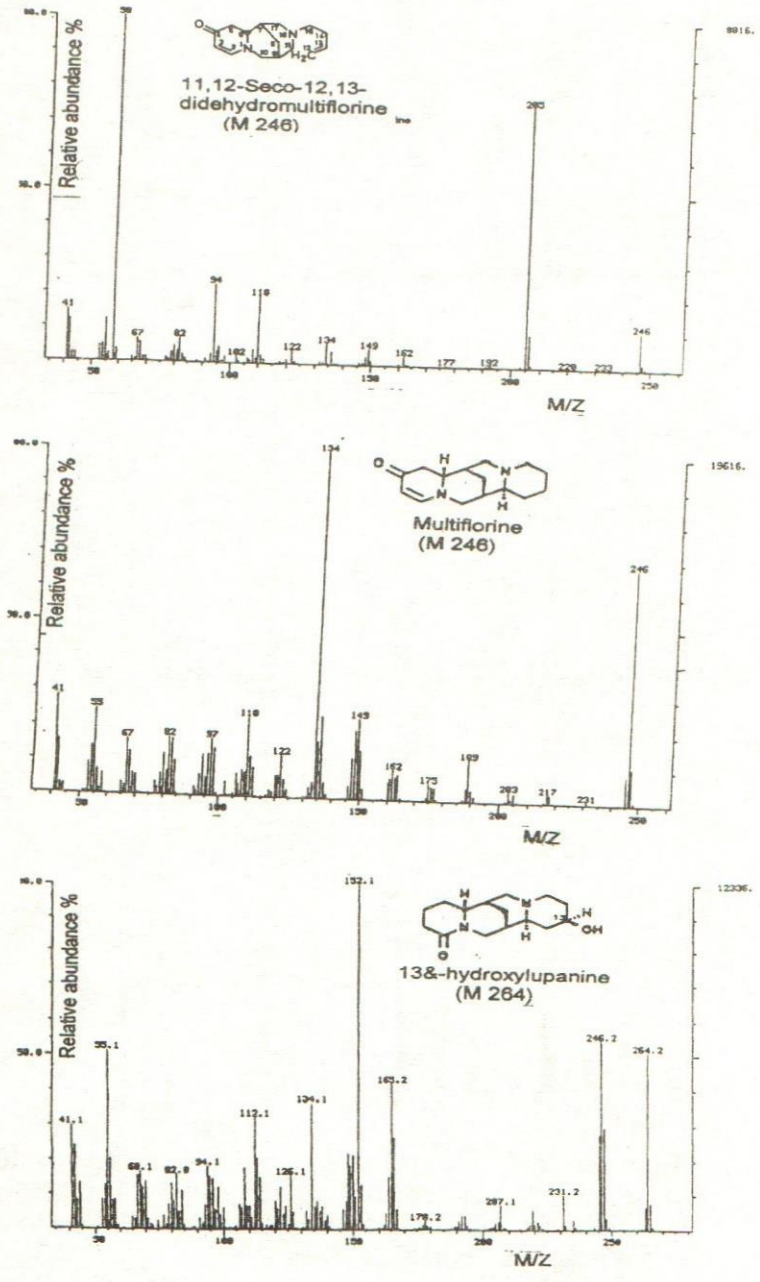


Figure (1): (Cont.).

4(16), 150(15), 94(8); Lupanine: RI =2165; MS: 136(100), 248(62), 149(61), 150(40), 55(38), 98(28); 11,12-seco-12,13-didehydromultiflorine: RI =2215; MS: 58(100), 205(75), 94(22), 110(20), 41(15), 246(11); Multiflorine: RI =2310; MS: 134(100), 246(68), 41(28), 55(25), 149(23), 110(22);13&-hydroxylupanine: RI =2400; MS: 152(100), 246(55), 264(51), 55(51), 165(41), 134(36).

Data presented in Table (1) show the components, retention times and percentages of individual alkaloids based on total alkaloids in seeds of heavy branching/high yield, early flowering mutants and control. It is clear that lupanine represents the major component (56.32-63.34%) in all investigated seeds followed by 13&-hydroxylupanine (12.40-17.32%) and multiflorine (8.64-13.74%). Whereas, albine (3.38-9.02%), angustifoline (4.09-6.75%) and 11,12-Seco-12,13-didehydromultiflorine (2.59-5.93%) represent the minor components.

Results of the individual alkaloids as mg/g seed dry weight are given in Table (2) and Figures (2, 3 and 4). It is realized that the concentration of all individual alkaloids were increased in seeds of heavy branching/high yield mutant (G2 EMS HB/HY6) as compared to the control. On the other hand, was found with (G2 SA H/HY3) mutant, which recorded an obvious decrease in all individual alkaloids compared to that of the control. The concentration of all individual alkaloids were increased in seeds of early flowering mutant (G2 SA EF2) as compared to the control except to multiflorine constitute was decreased by 24.60% less than that in seeds of the control. While, the concentrations of all individual alkaloids were decreased in seeds of early flowering mutant (G2 EMS EF1), except to angustifoline and 11,12-Seco-12,13-didehydromultiflorine constitutes were increased by 10.71%, 7.32% more than those of the control; respectively. It is noted that lupanine constitute recorded the highest value in seeds of heavy branching/high yield mutants (G2 EMS HB/HY6) and early flowering mutant `1(G2 SA EF2) being 65.07and 12.30% more than that in seeds of the control; respectively. On the other hand, it was decreased by 26.45%, 10.58% in seeds of heavy branching/high yield mutant (G2 SA HB/HY3) and early flowering mutant (G2 EMS EF1) less than that inseeds of the control; respectively. It is noted that the increase in total alkaloid content, which was observed in seeds of (G2 EMS HB/HY6), (G2 EMS EF1) and (G2 SA EF2), was reflected in increased concentration of individual components. Likewise, the reduction in the total content, which was observed in the(G2 SA HB/HY3), was reflected in reduced concentration of individual components (Figures,3 and 4). In this respect, Fartushnyak (1974) isolated useful mutations with low alkaloids content after treating seeds of *Lupinus albus* with chemical mutagens. The obtained mutants contained 0.001-0.03% alkaloids, compared with 0.25-2.8% alkaloids in the initial varieties, Jayasekera (1981) isolated mutants with low alkaloid content from *Lupinus albus* as well as from *Lupinus mutabilis*, a new mutant allele, described as mutal, which reduces the alkaloid content in dry matter of *Lupinus mutabilis* has been identified by Williams *et al.*(1984) following seed treatment with 0.04 and 0.06% EMS. The allele, when homozygous, reduced the alkaloid content from level of more than 2.0% found in seed dry matter of existing populations to 0.2 - 0.3% and produced plants with

Table (1): Alkaloids in seeds of heavy branching/high yield, early flowering mutants and control plants of lupine cv. Giza 2 in M7-generation, components, their percentages, and retention times

Mutant type	Alkaloids											
	1		2		3		4		5		6	
	Rt. (min.)	%	Rt. (min.)	%	Rt. (min.)	%	Rt. (min.)	%	Rt. (min.)	%	Rt. (min.)	%
Control (Giza 2)	8.87	3.38	10.86	4.09	11.85	59.82	12.37	3.04	13.41	13.74	14.40	15.93
G2EMS HB/HY6	8.78	9.02	10.78	4.61	11.80	61.98	12.30	2.59	13.34	9.40	14.32	12.40
G2 SA HB/HY3	8.77	4.51	10.75	4.95	11.72	62.93	12.26	3.42	13.28	11.41	14.23	12.77
G2 EMS EF1	9.22	3.60	11.13	5.39	12.09	63.34	12.60	3.87	13.60	9.17	14.54	14.63
G2 SA EF2	8.73	5.03	10.92	6.75	11.91	56.32	12.42	5.93	13.45	8.64	14.43	17.32

Table (2): Alkaloids content and composition in the seeds of heavy branching/high yield, early flowering mutants and control plants of lupine cv. Giza 2 in M7-generation

Mutant type	Alkaloid composition (mg/g seeds dry weight)						Total alkaloids content
	1	2	3	4	5	6	
Control (Giza 2)	0.46	0.56	8.13	0.41	1.87	2.16	13.59
G2 EMS HB/HY6	1.95	0.99	13.42	0.56	2.04	2.69	21.65
G2 SA HB/HY3	0.43	0.47	5.98	0.34	1.08	1.21	9.51
G2 EMS EF1	0.41	0.62	7.27	0.44	1.05	1.68	11.47
G2 SA EF2	0.82	1.09	9.13	0.96	1.41	2.81	16.22

1=Albine, 2=Angustifoline, 3=Lupanine, 4= 11,12-Seco-12,13-didehydromultiflorine
5= Multiflorine, 6= 13&- Hydroxylupanine

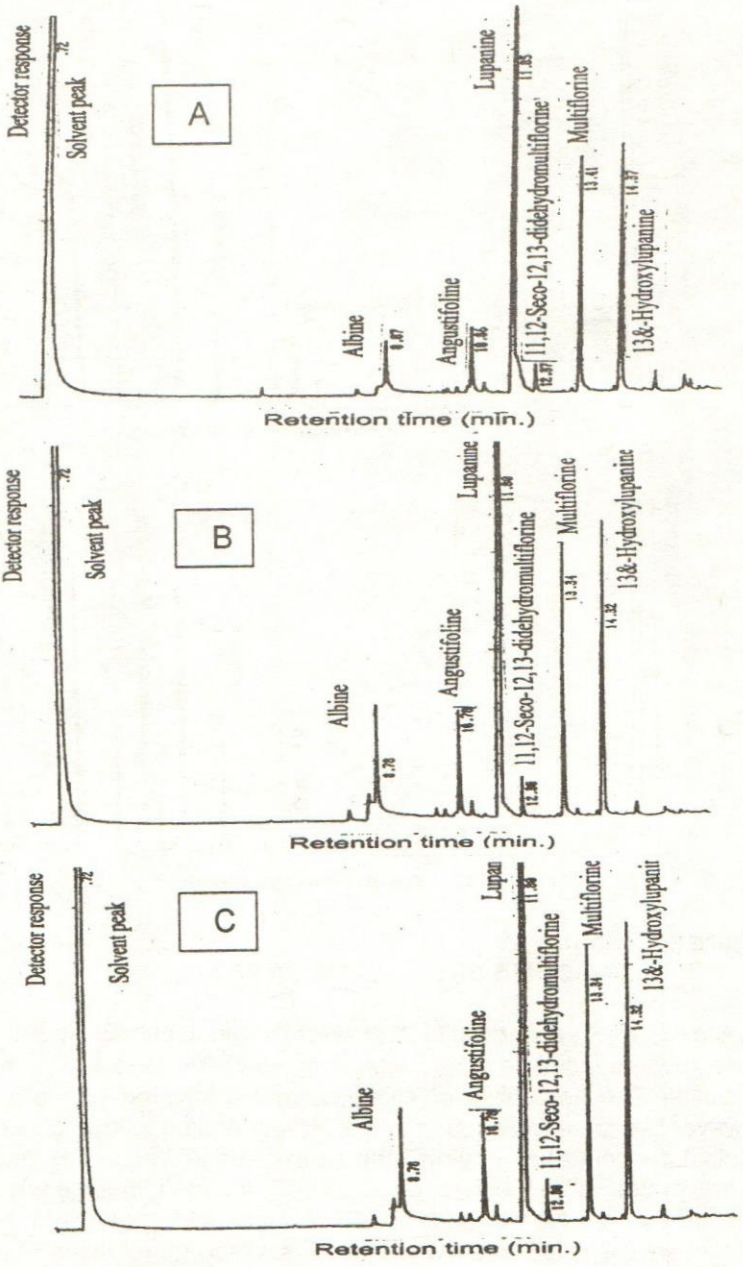


Figure (2): GLC analysis of alkaloids in seeds of heavy branching/high yield, early flowering mutants and control plants of lupine cv. Giza 2 in M7-generation.

A- Giza 2 (control). B- G2 EMS HB/HY6. C- G2 SA HB/HY3.

(Cont.)

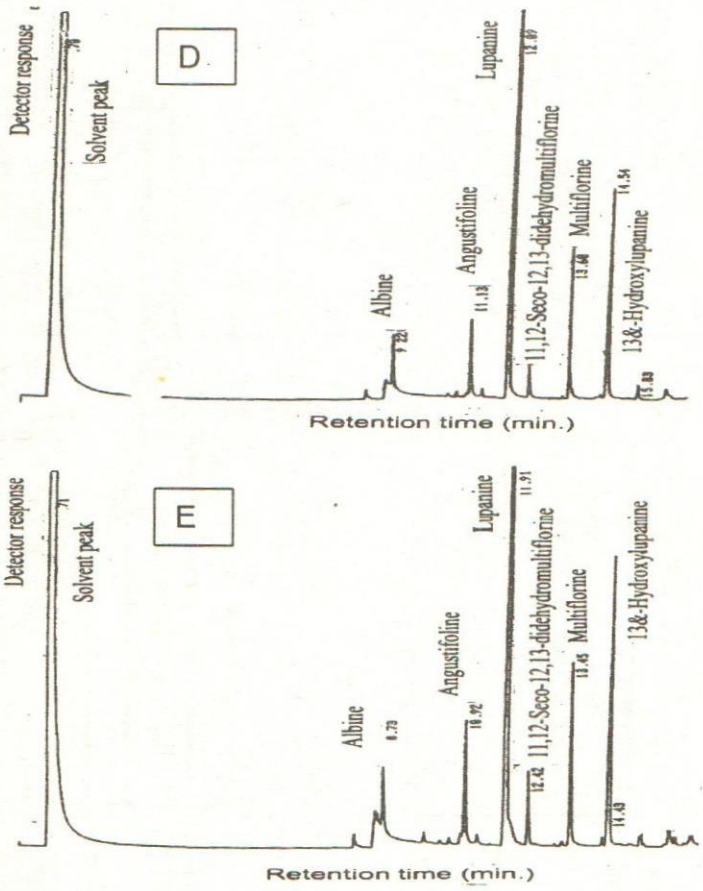


Figure (2): (Cont.).
D- G2 EMS EF1. E- G2 SA EF2.

vegetative and seed tissues that were organoleptically sweet. The mutant allele also reduced the percentage of sparteine and lupanine in the total alkaloids. The heritability of alkaloid content in bitter phenotypes was low; however two lines were found in the M5-generation following selection for low alkaloid concentration in which the concentration was in the range 0.2-0.8 % of dry matter and Klochko *et al.* (1992) induced mutagenesis in *Lupinus angustifolius* cv. Nemchinovskii 846. A high yield mutant with low alkaloidal content was isolated and evaluated for several generations. It was released as a new cultivar named (Ladnyi). The alkaloidal content in the seeds of such mutant ranged from 0.015 to 0.051 %. It was found that the reduction in the total alkaloid content was reflected in reduced concentration of individual components, being in agreement with the present findings.

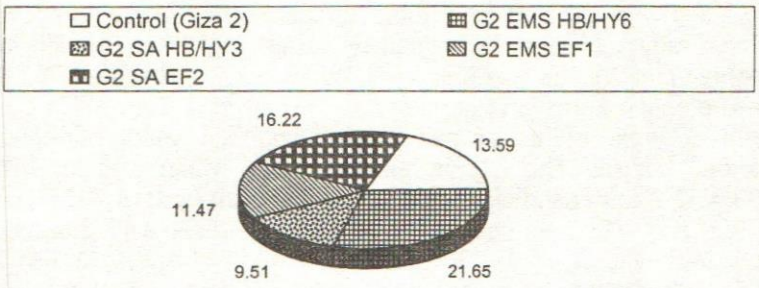


Figure (3): Total alkaloid content (mg/g seeds dry weight) in the seeds of heavy branching/high yield, early flowering chosen mutants and control plants of lupine cv. Giza 2 in M7-generation.

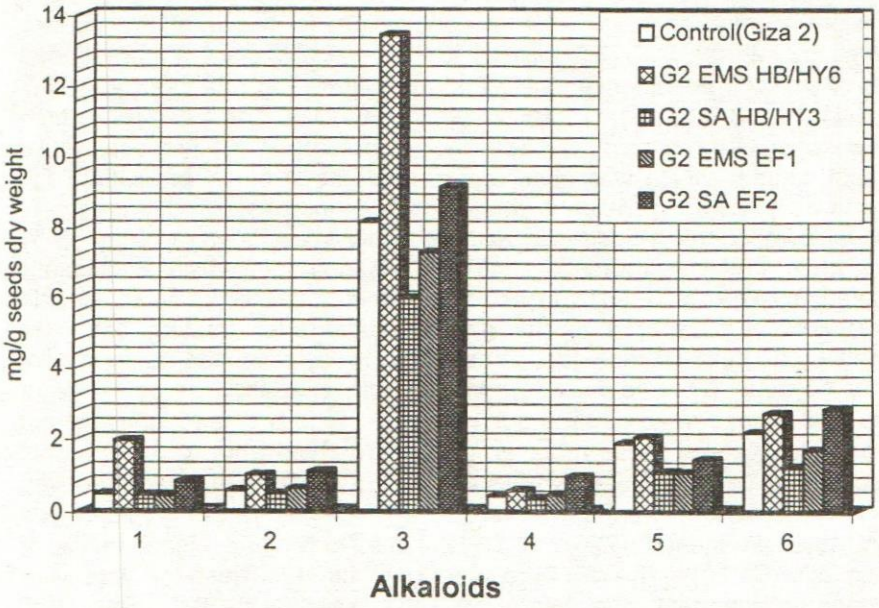


Figure (4): Alkaloid fractions in seeds of Egyptain lupine cv. Giza 2 and in seeds of M7-generation of heavy branching/high yield and early flowering mutants.

1=Albine, 2=Angustifoline, 3=Lupanine, 4=11,12-Seco-12,13-didehydromultiflorine, 5=Multiflorine, 6=13&-Hydroxylupanine

2- GLC analysis of fatty acids:

Fatty acids were studied chromatographically using GLC. As shown from data, seven fatty acids were identified by comparing their (Rt) with those of standard samples beside two unknown. While, those presented in Table (3) show the retention times and percentages of individual fatty acids based on total fatty acids in seeds of heavy branching/high yield, early flowering mutants and control. The higher relative concentration was for fatty acid C18:1(39.6-47.9%) in all investigated seeds, followed by C18:2 (21.4-24.8%), while C16:0 and C18:3 reached (16.6-18.2%) and (8.3-12.4%); respectively. The other fatty acids C18:0, C20:0, C22:0, unknown1 and unknown 2 were present in lesser amounts in all investigated seeds ranged from traces to 2.2%.

Results of the individual fatty acids as mg/g seed dry weight are found in Table (4) and Figures (5, 6 and 7). It is realized that the concentration of all individual fatty acids were increased in seeds of heavy branching/high yield and early flowering mutants as compared to the control. Whereas, fatty acid (C20:0) was present only in seeds of heavy branching/high yield mutant (G2 EMS HB/HY6) in lesser amounts (traces) 0.14 mglg dry weight. It is noted that fatty acid C18:1 constitute recorded the highest value in seeds of all heavy branching/high yield and early flowering mutants (20.74-25.33 mglg dry weight) compared to 17.75 mg/g dry weight in seeds of the control. Followed by fatty acids C18:2 , C16:0, C18:3 and C22:0 were increased in seeds of heavy branching/high yield and early flowering mutants by (30.67-101.90%), (10.73-66.31%), (11.48-142.30%) and (3.70-144.44%) more than those in seeds of the control; respectively. It is noted that the increase in total fatty acid content, which was observed in seeds of all heavy branching/high yield and early flowering mutants, was reflected in increased concentration of individual components (Figures, 6 and 7). Similar results were obtained by Wilcox *et al.* (1984) a mutant C1640 in M4-generation, which significantly increased in oleic acid (C18:1) content and decreased in linolenic acid (C18:3) content compared to the parent variety after treating seeds of soybean by a chemical mutagen, Graef *et al.*(1985) produced three mutant lines of soybean (*Glycine max* (L.) Merrill) with increased percentages of stearic acid in their seed oil of about 16 to 30% (3 to 6 times higher than in their parents). One of these mutants (A6) was derived from seed treatment with sodium azide(NaN3) and two (FA 41545 and A 81-606085) were derived from seed treatment with EMS, Wilcox and Cavins(1990) obtained two mutant soybean lines (C1726 and C1727) had lower and higher levels of palmitic acid (C16:0) in the oil of the seed; respectively. These mutants were developed by treatment of soybean cv. Century seeds with EMS, Fehr *et al.* (1991) obtained two mutant soybean lines Elgin EMS-421 and A 1937 NMU-85 were developed by treatment of Elgin seeds with EMS and A 1937 seeds with NMU. These mutants had elevated palmitic acid content in their seed oil, Rahman *et al.*(1994) obtained a mutant line B739 had higher level of linolenic acid content in its seed oil. This line was identified from the soybean(*Glycine max* cv. Bay) in a mutation breeding programme, Osorio *et al.*,(1995) isolated four mutants after treating seeds of sunflower (*Helianthus annuus L.*) with

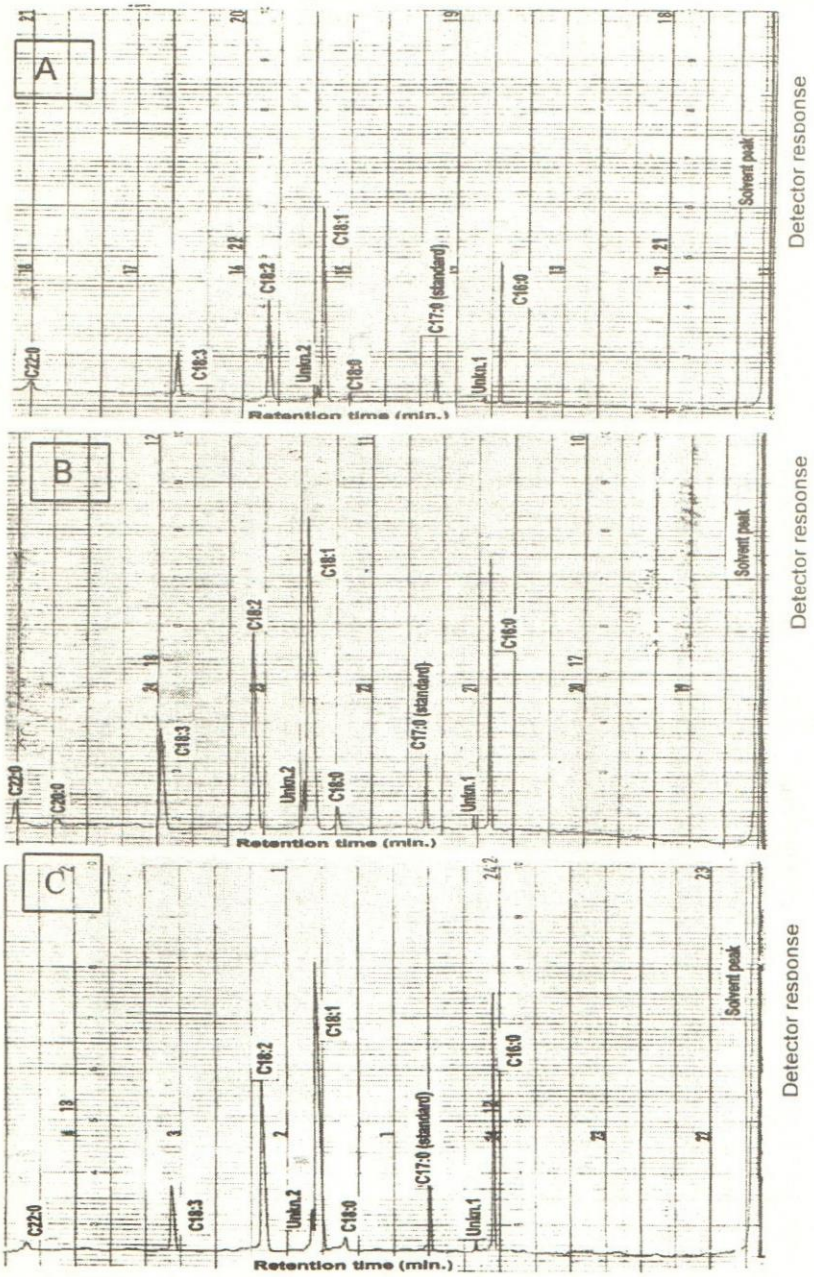
Table (3):Fatty acids in seeds of heavy branching/high yield, early flowering mutants and control plants of lupine cv. Giza 2 in M7-generation, components, their percentages, and retention times

Mutant type	Fatty acids																	
	C16:0		Unk.1		C18:0		C18:1		Unk.2		C18:2		C18:3		C20:0		C22:0	
	Rt. min.	%	Rt. min.	%	Rt. min.	%	Rt. min.	%	Rt. min.	%	Rt. min.	%	Rt. min.	%	Rt. min.	%	Rt. min.	%
Control (Giza 2)	15.3	17.9	16.4	0.3	23.9	1.1	25.4	47.9	25.8	1.1	28.5	21.4	33.6	8.9	--	--	42.0	1.4
G2EMS HB/HY6	15.4	17.1	16.4	0.5	24.1	1.2	25.7	39.6	26.0	2.2	28.7	24.8	33.9	12.4	39.9	0.2	42.3	0.2
G2 SA HB/HY3	15.3	16.8	16.3	0.3	23.7	1.3	25.4	45.0	25.5	1.4	28.3	23.8	33.3	9.3	--	--	41.7	2.1
G2 EMS EF1	15.3	16.6	16.4	0.3	23.9	1.5	25.5	46.9	25.7	0.8	28.5	24.6	33.6	8.3	--	--	41.9	1.0
G2 SA EF2	15.4	18.2	16.5	0.4	24.1	1.5	25.6	45.6	25.9	1.8	28.7	21.6	33.8	9.5	--	--	42.2	1.4

Table (4):Fatty acids composition in the seeds of heavy branching/high yield and early flowering mutants and control plants of lupine cv. Giza 2 in M7-generation

Type of Mutants	Fatty acids (mg/g dry weight)								Total fatty acids content
	C16:0	Unk.1	C18:0	C18:1	Unk.2	C18:2	C18:3	C20:0	
Control (Giza 2)	6.62	0.12	0.44	17.75	0.44	7.89	3.31	--	0.54	37.11
G2 EMS HB/HY6	11.01	0.31	0.83	25.33	1.46	15.93	8.02	0.14	1.32	64.35
G2 SA HB/HY3	9.05	0.21	0.71	24.44	0.79	12.79	5.03	--	1.16	54.27
G2 EMS EF1	7.33	0.15	0.71	20.74	0.37	10.85	3.69	--	0.56	44.27
G2 SA EF2	8.71	0.22	0.71	21.74	0.88	10.31	4.52	--	0.66	47.75

C16:0 = Palmitic acid, Unk.1=Unknown1, C18:0 = Stearic acid, C18:1 = Oleic acid, Unk.2=Unknown2, C18:2 = Linoleic acid, C18:3 = Linolenic acid, C20:0 = Arachidic acid , C22:0 = Behenic acid



Figure(5): GLC analysis of fatty acids in seeds of heavy branching/high yield, early flowering mutants and control plants of lupine cv. Giza 2 in M7-generation.
A- Giza 2 (control). B-G2 EMS HB/HY6. C- G2 SA HB/HY3.
(Cont.)

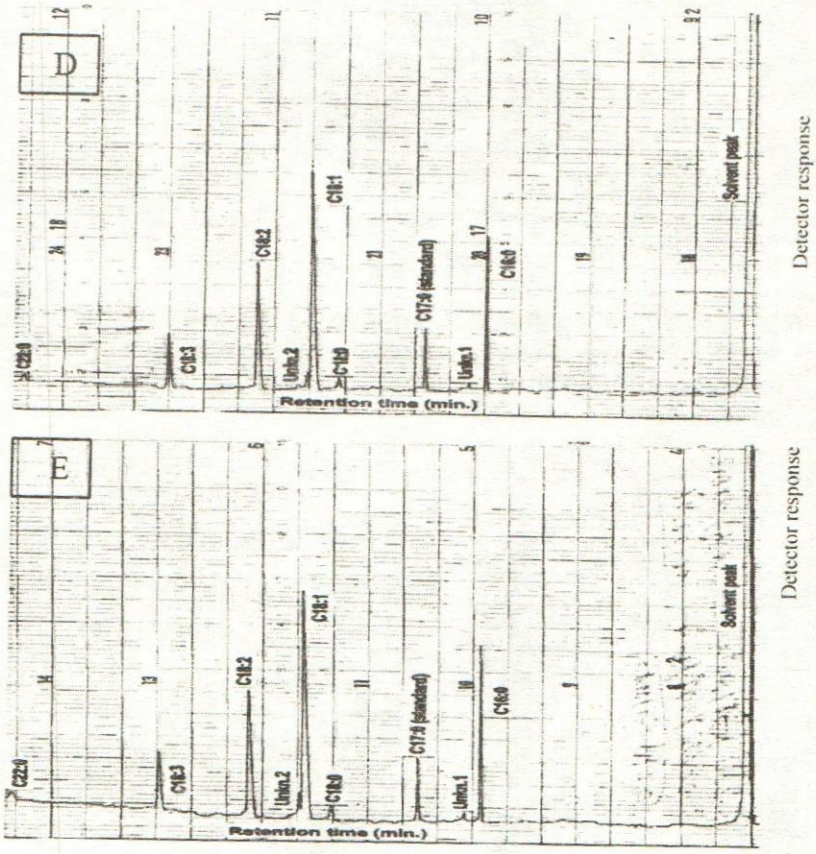


Figure (5): cont.
D- G2 EMS EF1. E- G2 SA EF2.

EMS, SA and X-radiation. The mutant lines included CAS5, which has oil characterized by a 5-fold increase in palmitic acid (C16:0), and CAS3, CAS4 and CAS8, which have from 2 to 6 times the stearic acid(C18:0) content higher than in their parents. All, being in accordance with the present findings.

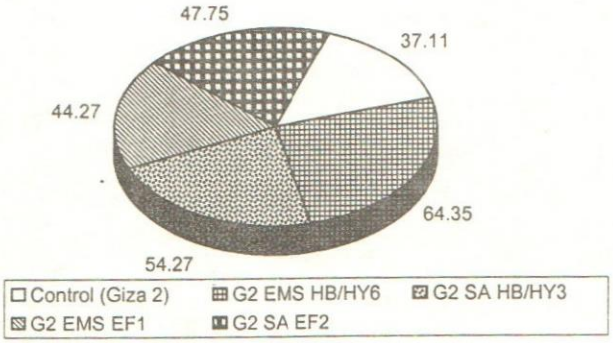


Figure (6): Chloroform soluble lipids (mg/g seeds dry weight) in the seeds of heavy branching/high yield, early flowering chosen mutants and control plants of lupine cv. Giza 2 in M7-generation.

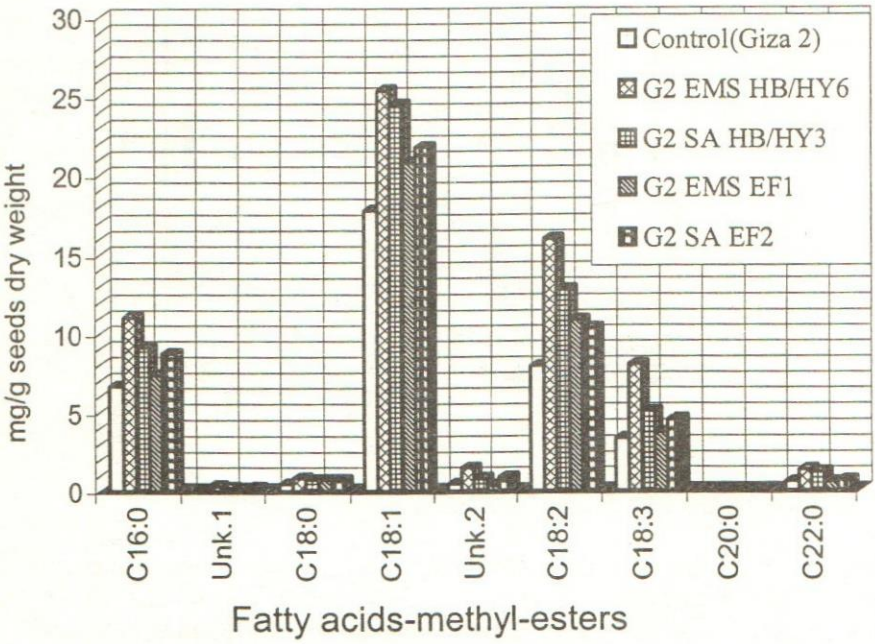


Figure (7): Fatty acids composition in the seeds of heavy branching/high yield, early flowering chosen mutants and control plants of lupine cv. Giza 2 in M7-generation.

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تقييم طفرات مبكرة التزهير و غزيرة التفريع/عالية المحصول فى الأجيال المتقدمة من الترمس المصرى

٢-الخصائص الكيميائية للطفرات

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اشتملت المواد النباتية المستخدمة فى هذه الدراسة على أربع طفرات من الترمس صادقة التوالد تنتمى إلى طرازين مختلفين من الطفرات (٢ مبكرة التزهير و ٢ غزيرة التفريع / عالية المحصول) اشتقت من الترمس المصرى صنف جيزة ٢ كنتيجة لمعاملة بذوره بأي من المطفرين الكيميائيين ايثايل ميثان سلفونات (EMS) و الصوديوم آزايد (SA) و ذلك خلال برنامج للتربية باستحداث الطفرات (حسن، ١٩٩١ و ١٩٩٨). تم تقييم هذه الطفرات فى الجيل السابع لدراسة خصائصها الكيميائية من القلويدات والأحماض الدهنية للزيت.

برهن تحليل GLC على وجود ست قلويدات فى بذور الترمس المصرى صنف جيزة ٢ والطفرات تحت الدراسة. حيث سجل ال Lupanine أكبر محتوى بنسبة (٥٦,٣٢-٦٣,٣٤%) فى كل البذور تحت الدراسة، يليه 13&-hydroxylupanine و الذى سجل (١٢,٤٠-١٧,٣٢%) ، يليه Multiflorine بنسبة (٨,٦٤-١٣,٧٤%). بينما سجل كلا من Albine (٣,٣٨-٩,٠٢%) ، Angustifoline (٤,٦١-٦,٧٥%) ، 11-12-Seco-12-13-didehydromultiflorine (٢,٥٩-٥,٩٣%) و التى وجدت كمكونات صغرى. من الملاحظ أن الزيادة فى محتوى القلويدات الكلية فى بذور الطفرة غزيرة التفريع/عالية المحصول (G2 EMS HB/HY6) و مبكرة التزهير (G2 EMS EF1) و(G2 SA EF2) يرجع إلى زيادة تركيز المكونات الفردية للقلويدات. كما أن النقص فى محتوى القلويدات الكلية فى بذور الطفرة غزيرة التفريع/عالية المحصول (G2 SA HB/HY3) يرجع الى نقص تركيز المكونات الفردية للقلويدات.

أيضا برهن تحليل GLC على وجود سبعة أحماض دهنية بالأضافة الى وجود مكونين مجهولين فى كل البذور تحت الدراسة. حيث سجل الحامض الدهنى C18:1 أكبر محتوى بنسبة (٣٩,٦-٤٧,٩%) فى كل البذور تحت الدراسة متبوعا بالحامض الدهنى C18:2 و الذى سجل (٢١,٤-٢٤,٨%). بينما سجل كلا من الحامض الدهنى C16:0 و C18:3 نسبة (١٦,٦-١٨,٢%) ، (٨,٣-١٢,٤%) على التوالى. بالإضافة إلى الأحماض الدهنية C18:0 و C20:0 و C22:0 و المكونين المجهولين و التى تراوحت نسبتها بين آثار قليلة إلى٢,٢ %. من الملاحظ أن زيادة المحتوى الكلى للأحماض الدهنية فى بذور الطفرات غزيرة التفريع/عالية المحصول و مبكرة التزهير يرجع الى زيادة تركيز المكونات الفردية للأحماض الدهنية.