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CHEMICAL MUTAGEN INFLUENCE IN THE IMPROVEMENT OF SOME PEA AGRONOMIC AND BIOCHEMICAL CHARACTERS

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ABSTRACT

During two successive seasons (2003-2004) and (2004-2005), pea seeds of two cultivars, Master B and Lincoln, were soaked under 0.001, 0.002, 0.003, 0.004 sodium azide concentrations for one, two and three hours for each concentration. Split split plot design with four replicates was used. The result were as follow:

The first season (2003-2004)

1- Vegetative characters:

In the first generation (M_1)of Master B cultivar. 0.002 concentration of sodium azide for one hour gave the highest significant values (93.75%, 68.38cm, 21.55 and 3.26) with increasing 15.88% , 82.69% , 57.07 , and 100.87%) over the parent Master B for germination ratio, Plant height , number of leaves and branches per plant , respectively, while 0.004 concentration for two hours decreased number of leaves and branches by 21.87% and 22.58%. The same dose but for three hours reduced both germination ratio by 17.52% and 14.01% for plant height.

In Lincoln M_1 generation, 0.002 concentration for two hours gave the highest values of germination ratio (94.78%), plant height (98.13cm), and 4.88 for number of branches per plant. Inseasing the time of soaking to three hours, same concentration gave (24.40) as the highest significant number of leaves, with increase 12.39%, 50.21%, 64.53% and 92.89% for vegetative characters, respectively. The depression under 0.004 concentration for three hours was 17.48%, 23.91%, 24.48% and 63.21% for the same characters, respectively.

2- Yield and it's components

In Master B, M_1 generation, 0.001 sodium azide concentration for two hours gave the highest significant values (25.67) for number of pods, (205.18) for number of seeds and 430.11 (g) for yield of green pods per plant with an increase 144.94%, 174.67% and 148.76% for the same characters, respectively. Revers results were obtained under 0.004 for three hours. Moreover, in Lincoln M_1 generation, the predictable concentration was 0.002 sodium azide for three hours, that gave highly significant values (28.83, 198.93, and 410.63 (g)for number of pods, seeds per plant, and yield of green pods, with an increase 114.35%, 103.65% and 81.99%. 0.004 of sodium azide concentration causes reduction, 33.09%, 48.30% and 59.82%, for the same characters, respectively.

The same trend of results was obtained in the second season (2004-2005) for vegetative characters, yield and it's components.

3- Biochemical Studies:

Protein electrophoretic studies, showed that low sodium azide concentrations "0.001 and 0.002" in the M_1,s of either Master B or Lincoln were distinguished with the highest protein band number and intensity and exceeded all the remaining treatments as well as their respective parents. The same results look like and near to that obtained by hybridization methods. Moreover same concentrations "0.001 and 0.002" sodium azide gave the highest increase in protein content by 16.73% and 13.07% for M_1 of Master B and Lincoln, respectively.

INTRODUCTION

The improvement of pea yield capacity, without time waste and hard work in traditional hybridization, considered an important process for breeding this crop. The cultivars may have different and quite sensitivity to chemical mutagen such as sodium azide. Such differences in their response may be due to the sensitivity of different genes to the chemical mutagens Azza (2004). Thus this reflects on the behavior of some agronomic characters.

The important source of protein in pea seeds made this crop ranking an important position as one of the four most important grain legumes in the world. So, studying the mutability of some pea cultivars differ in their sensitivity to chemical mutagen is favorable in our study.

Producing high yielding potential in M_1 generation with high protein content was carried out by many researches i.e. Reddy (1992); Mihov *et al* (2001) and Naglaa (2001).

Geetha & Vaidianathan (1998) and Naglaa (2001) also, pointed out that genetic variation caused by the mutagens are favorable if it will be related to some agronomic characters "yield and some of it's components":

The aim of this investigation is how to utilize of the mutagenesis in pea breeding and production through the elevation of sodium azide mutagen to some agronomic and biochemical pea characters.

MATERIAL AND METHODS

This work was carried out through two successive seasons (2003-2004) and (2004-2005) at a private farm in Sharkia Governorate to study the influence of chemical mutagens "sodium azide" under different concentrations on some pea agronomic characters in the first mutagenic generation of Master B and Lincoln cultivars, that were obtained from Hort. Res. Institute. Egypt and differed in their growth habit.

Preparation of sodium azide solution:-

The stock solutions were prepared by dissolving 3.25 g. sodium azide (NaN_3) in 50 ml. distilled water to obtain one mole sodium azide solution. Phosphate buffer solution was prepared by dissolving 12g potassium phosphates ($KH_2 PO_4$) in one liter of distilled water. Phosphoric acid ($H_3 PO_4$), was slightly added, drop by drop, to the above buffer solution to reach pH_3 consulting pH- meter.

The concentrations of 0.001, 0.002, 0.003 and 0.004 Mole were prepared by adding 1.0 ml. and 2.0, 3.0 and 4.0 ml from stock solution to one liter flask for each concentration and diluting up to 1000 ml. using phosphate buffer solution. 400 seeds (per-treatment) for each variety were soaked in each concentration for the three times of soaking at 20 C^0 i.e one, two and three hours. A control material of 400 seeds for each variety soaked in distilled water under the same conditions was maintained for comparison with the various mutagenic treatments. The treatments are concentrations of sodium azide as follow.

1-Distilled water : 2- 0.001
3- 0.002 4- 0.003 5- 0.004

Each concentration was soaked for one, two and three hours as three times of soaking.

Pea seeds of both Master B and Lincoln were sown in hills "15 cm a part" in rows with 10 m length for each row in Oct. 15th. Fertilization, irrigation and other field practices of growing field pea in Egypt were carefully followed up to full maturity, according to the guidance of Ministry of Agriculture.

A spilt split plot design with four replicates was used for sodium azide experiment where as, the cultivars were distributed at the main plots, while sodium azide levels were distributed randomly at the sub plots and the time of soaking (hours) in sub sub plots. Data on 40 plants, represented by 10 plants from each replicate and each treatment were as follow:-

1) Vegetative characters:-

a)Germination ratio b)Plant height (cm)

c) Number of leaves per plant. d)Number of branches per plant.

2) Yield and some of it's components

a)Number of pods per plant b) Number of seeds per plant.

c) Yield of green pods per plant (g)

Data were subjected to the proper statistical analysis of variance according of Snedecor and Cochran (1982). L.S.D at 5% and 1% levels of significance was used to compare treatment means.

Biochemical studies

A- Protein electrophoresis

This investigation was carried out at Laboratory of Genetic Engineering. Department of Genetics. Faculty of Agriculture. Ain Shams University. Sodium dodocyl sulphate – polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method being modified by Studier (1973).

Sample preparation:

Seed samples from the first generation mutagenic and the two pea cultivars "Master B and Lincoln" were used. Seeds were pressed by a drill to repture the cells and release their contents. Samples of 0.5 g of each genotype with 5 ml of sample buffer were homogenized, then they were containing water soluble protein and used for SDS-PAGE.

Gel preparation:

Polyacrylamide standard gel at pH 8.9 consists of 150 ml monomer solution (8.55 Acrylamide; 0.45 Bisacrylamide in 0.150 M Tris-Borate buffer). Then the following were added without delay: 300 mgs sodium sulphate (dissolve completely); 0.40 ml TEMED (tetramethlendiamine), and 40 ml ammonium Presulphate (2%) freshly prepared 200 μ extract of each sample was mixed with 50 μ glecrol and 50 μ bromophenol blue.

Gel incubation and agitation were carried out at room temperature until the bands appear in clear background. Then the gel was washed with distilled water and photographed.

B- Protein Chemical analysis:

A random sample of dry seeds (0.500 g) was taken from each treatment and finally ground. The samples were digested to estimate nitrogen, using the methods outlined by Kock and McMeekin (1924).

RESULTS AND DISCUSSION

A- The first season (2003-2004)

1-Vegetative characters:

The effect of cultivars, sodium azide concentrations and time of soaking on some vegetative characters is shown in Table (1). Data show that Lincoln cv has the best values with significant differences. This result ensures that the two cultivars "Lincoln and Master B" differ in their growth habit. Moreover, the concentration "0.002" of sodium azide for two hours of soaking gave the best effect.

Table (1): Effect of cultivars, sodium azide concentrations and time of soaking (h), on some vegetative characters through two successive seasons (2003-2004) and (2004-2005).

Main effects	Germination ratio		Plant height (cm)		N. of leaves /plant		N. of branches / plant	
	First Season	Second Season	First Season	Second Season	First Season	Second Season	First Season	Second Season
Cultivars (C)								
Master B	82.90	83.40	40.25	41.67	10.33	10.16	1.66	1.63
Lincoln	85.01	84.63	86.17	88.50	14.98	15.36	2.58	2.71
L.S.D 0.05	1.23	1.09	2.67	2.83	0.06	0.17	0.08	0.07
Sodium azide (S) concentrations								
0.00	81.88	82.07	68.73	70.03	13.77	14.06	2.01	2.08
0.001	87.66	88.13	70.56	72.19	16.79	15.68	2.50	2.63
0.002	92.07	92.11	85.67	82.09	19.55	20.17	3.12	3.36
0.003	80.05	80.33	64.77	65.33	14.61	14.50	1.78	1.83
0.004	74.22	73.67	58.88	57.15	11.33	10.67	1.13	1.07
L.S.D 0.05	1.18	1.26	1.36	1.17	1.63	1.44	0.06	0.09
Time of soaking (h)								
1 h	82.76	82.33	62.64	62.50	13.51	14.78	2.10	2.20
2 h	90.32	91.81	70.55	68.17	15.48	15.53	2.64	2.39
3h	80.11	79.67	60.96	61.88	14.62	13.98	2.06	2.18
L.S.D 0.05	1.16	1.19	2.03	2.11	0.17	0.21	0.07	0.06
C × S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S
C × h	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S
S × h	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S

Table (2) clearly shows that the M_1 generation derived from soaking the cultivar Master B with predictable dose of sodium azide "0.002 for two hours gave the highest significant values (93.75% , 68.38cm , 21.55 and 3.16) for germination ratio , plant height, number of leaves and number of branches per plant, respectively. The slight difference between the two soaked cultivars Master B and Lincoln in sensitivity and the degree of response were observed under "0.002" sodium azide concentration. In such predictable concentration increasing the time of soaking for three hours gave only the highest significant number of leaves per plant (24.4) in Lincoln, while this dose for two hours activated germination ratio, plant height and number of branches.

Table (2) Some vegetative characters of M_1 generation pea plants as affected by the interaction among cultivars, sodium azide concentrations and time of soaking (hours) through two successive seasons (2003-2004) and (2004-2005)

Cultivars	Sodium azide concentration	Time of soaking (hours)	Germination ratio		Plant height(cm)		No. of leaves /plant		No. of branches / plant	
			First season	Second season	First season	Second season	First season	Second season	First season	Second season
Master B	Control	1h	80.50	81.05	36.40	37.00	10.93	11.03	1.30	1.36
		2h	80.90	80.70	37.43	38.11	13.72	13.80	1.59	1.67
		3h	85.00	84.83	40.83.	41.63	12.48	12.60	1.50	1.63
	0.001	1h	86.00	86.77	46.38	47.30	15.73	15.8	2.58	2.60
		2h	88.77	89.63	66.77	63.81	20.48	21.5	3.13	3.16
		3h	84.83	83.90	51.95	50.70	18.08	18.0	2.75	2.83
	0.002	1h	87.50	86.30	50.00	49.81	15.93	16.0	2.05	2.15
		2h	93.75	94.50	68.38	69.03	21.55	23.0	3.16	3.26
		3h	90.50	90.42	51.88	50.71	14.73	14.8	2.00	2.01
	0.003	1h	80.33	80.11	50.98	50.87	17.00	17.07	1.78	1.86
		2h	80.77	79.82	45.77	44.66	15.55	15.31	1.70	1.73
		3h	80.00	78.73	43.11	42.70	13.60	13.11	1.63	1.59
	0.004	1h	76.51	76.00	40.50	40.0	13.00	13.05	1.50	1.53
		2h	75.66	76.02	38.33	38.60	10.72	10.69	1.20	1.21
		3h	70.11	78.73	35.11	34.70	10.11	10.00	1.03	1.02
Lincoln	Control	1h	83.50	83.12	70.48	71.08	14.46	14.51	2.40	2.41
		2h	84.33	84.4	65.33	65.78	14.66	14.70	2.53	2.54
		3h	83.70	83.86	75.70	76.03	14.83	15.07	2.80	2.78
	0.001	1h	85.36	85.45	85.40	85.11	16.43	16.5	3.80	3.78
		2h	86.77	87.00	86.65	87.00	15.40	15.6	2.90	2.88
		3h	86.12	86.29	85.68	86.1	15.36	15.29	2.80	2.80
	0.002	1h	88.33	88.11	89.03	89.18	17.45	17.60	3.90	3.88
		2h	94.78	95.67	98.13	98.00	18.50	18.40	4.88	4.90
		3h	88.36	88.55	97.35	97.11	24.40	25.2	3.33	3.40
	0.003	1h	81.71	82.00	81.95	81.36	14.58	14.6	2.05	2.03
		2h	80.07	80.37	86.50	86.00	13.48	13.53	1.88	1.89
		3h	80.00	80.05	80.45	80.01	13.37	13.40	1.76	1.75
	0.004	1h	77.62	76.67	65.20	66.12	12.23	12.3	1.05	1.04
		2h	76.32	75.43	62.60	63.00	12.23	12.33	1.02	1.01
		3h	69.07	68.11	57.60	58.7	11.20	11.4	1.03	1.02
	L.S.D 0.05		0.07	0.08	0.56	0.67	0.30	0.32	0.01	0.02
	L.S.D 0.01		0.36	0.43	0.73	0.83	0.57	0.61	0.17	0.10

with highly significant values (94.78%, 98.13 cm and 4.88), respectively. On the other hand, the increase in sodium azide concentrations reduced significantly all vegetative characters.These results agree with those obtained

by Dekov (1976) who found that increasing doses of ethylene imine reduce stem height in some durum wheat varieties, moreover Hajduch *et al* (1999) proved that more than one Mm sodium azide increased number of branches in some soybean cv. Naglaa (2001) reported that the high concentration, "0.25%" of ethylene imine reduced significantly plant height and number of tillers in triticale and added the presence of significant interaction between triticale strains and chemical mutagen treatments, in M_1 generation. On the other hand Rehman *et al* (2001) in *vigna mungo L.*, recorded a dwarf mutant as a the effect of 0.03% concentration of hydrazyne hydrate as a chemical mutagen.

As for the elevation and depression of some vegetative characters , (Table 3), shows that 0.002 concentration of sodium azide for two hours increased germination ratio plant height, number of leaves and number of branches per plant by 15.88%, 82.69% 57.07% and 103.87%, respectively in Master B cultivar. At the contrary, 0.004 concentration of sodium azide for two hours gave the highest percentage of depression (21.87% and 22.58%) for number of leaves and branches, while soaking for three hours gave 17.52% and 14.01% depression against the control for germination ratio and plant height.

Table (3) Elevation and depression, in some vegetative characters under sodium azide mutagen in pea M_1 generation through two successive seasons (2003-2004)and (2004-2005) .

Cultivars	Sodium azide concentrations	Time of Soaking (hours)	Germination ratio		Plant Height (cm)		No – of Leaves/ Plant		No. of branches/ Plant	
			First season	Second season	First season	Second season	First season	Second season	First season	Second season
Master B	0.001	1h	6.36	7.06	27.42	24.84	43.92	43.26	98.46	91.18
		2h	9.73	11.07	78.39	67.44	49.27	55.8	101.94	98.22
		3h	-0.2	-1.1	26.86	22.32	44.87	42.86	83.33	73.62
	0.002	1h	2.48	7.06	7.76	34.62	64.04	45.06	57.69	58.09
		2h	15.88	17.10	82.69	81.13	57.07	66.67	103.87	95.21
		3h	6.46	6.60	27.06	21.78	34.77	17.46	33.33	23.31
	0.003	1h	-0.21	- 1.16	40.06	37.05	55.54	54.76	36.92	36.76
		2h	-0.16	- 1.09	22.28	17.19	13.34	11.55	9.68	3.59
		3h	-5.88	- 7.66	5.58	2.57	8.97	14.05	8.67	2.45
Lincoln	0.004	1h	-4.96	- 6.23	11.26	8.11	18.94	18.31	15.38	12.50
		2h	-6.48	- 5.80	2.4	1.29	-21.87	-22.54	-22.58	-27.54
		3h	-17.52	- 17.33	-14.01	- 16.65	-18.99	-20.63	-20.77	-37.42
	0.001	1h	5.78	2.80	21.17	19.74	13.62	13.71	58.33	56.85
		2h	2.98	3.08	32.63	32.26	5.08	6.12	14.62	13.39
		3h	2.91	2.90	13.18	13.24	3.57	1.46	0.14	0.72
	0.002	1h	5.78	6.00	26.32	25.46	20.68	21.3	62.50	61.00
		2h	12.39	13.00	50.21	48.98	26.19	25.17	92.86	92.91
		3h	5.57	5.59	28.60	27.24	64.53	67.22	20.65	22.30
	0.003	1h	-2.14	- 1.35	16.27	14.46	0.82	0.62	-14.58	-15.77
		2h	-5.05	- 4.77	32.40	30.74	-8.05	-7.96	-25.69	-25.59
		3h	-4.42	- 4.54	6.27	5.23	-9.84	-11.08	-36.3	-22.30
	0.004	1h	-7.04	- 7.76	-7.49	- 6.98	-15.42	15.23	-56.25	-56.85
		2h	-9.50	- 10.63	-4.18	- 4.23	-16.58	-16.12	-59.68	-60.24
		3h	-17.48	- 18.78	-23.91	- 22.86	-24.48	-24.35	-63.21	-63.31

In Lincoln cultivar (12.39%, 50.21% and 92.86%) increase over the control were determined from 0.002 dose for two hours, concerning germination ratio , plant height and number of branches , respectively. Moreover three hours soaking gave 64.53% increase over the control for number of leaves per plant. Under the dose "0.004" for three hours, germination ratio, plant height, number of leaves, and number of branches show 17.48%, 23.91, 24.48% and 63.21% depression, respectively. These results agree with those reported by Atia *et al* (1995) and Azza (2004).

2. Yield and some of it's components:-

Data in Table (4) elucidate that Lincoln c.v has the best effect on yield and some of it's components. Moreover the two predictable doses of sodium azide "0.001 and 0.002" recorded also the superior values in the same characters with no significant differences. On the other hand, the two times of soaking "one and two hours" gave the best effect with insignificant differences.

Table (4): Effect of cultivars, sodium azide concentrations and time of soaking (h), on yield and some of it's component during two successive seasons (2003 – 2004) and (2004 – 2005).

Main effects	N. of pods / plant		N. of seeds / plant		Yield of green pods (g) / plant	
	First Season	Second Season	First Season	Second Season	First Season	Second Season
Cultivars (c)						
Maste B	10.60	10.34	72.50	70.34	166.73	158.68
Lincoln	13.09	13.27	88.66	88.27	207.30	201.69
L.S.D 0.05	1.06	1.08	4.66	4.9	6.77	7.13
Sodium azide concentrations						
0.000	11.03	10.66	80.58	79.21	186.96	180.19
0.001	18.05	19.17	121.56	118.68	251.45	243.40
0.002	18.42	19.00	120.67	118.01	251.59	249.59
0.003	11.63	12.06	79.87	68.37	163.24	158.03
0.004	9.53	9.50	53.63	52.78		
L.S.D 0.05	1.03	1.16	4.70	4.33	6.06	7.13
Time of soaking (h)						
1 h	13.56	13.66	86.28	84.86	181.57	176.90
2 h	14.38	14.51	96.35	94.37	203.70	198.05
3 h	14.02	14.79	94.16	92.36	192.00	183.18
L.S.D 0.05	0 35	0.20	3.19	3.27	11.65	10.33
C × S	N.S	N.S	N.S	N.S	N.S	N.S
C × h	N.S	N.S	N.S	N.S	N.S	N.S
S × h	N.S	N.S	N.S	N.S	N.S	N.S

From Table (5), it could be clearly observed the difference of sensitivity between the two studied cultivars "Master B and Lincoln" for the degree of response to the chemical mutagen sodium azide. The M_1 of Master B was sensitive to the predictable dose 0.001 for two hours, while 0.002 concentration for three hours activated yield and some of it's studied component in Lincoln cultivar. In Master B the optimum dose "0.001" sodium azide for two hours gave the highest significant values of number of pods per plant (25.67), number of seeds per plant (205.18), and yield of green pods (430.11) g.

Table (5): Yield and some of it's components of M_1 generation pea plants as affected by the interaction among cultivars, sodium azide concentrations and time of soaking, through two successive seas ons (2003-2004) and (2004-2005).

Cultivars	Sodium azide concentr-ations	Time of soaking (hours)	No. of pods / plant		No. of seeds / plant		Yield of green pods / plant (g)	
			First season	Second season	First season	Second season	First season	Second season
Master B	Distilled water	1h	9.28	9.53	61.88	60.33	143.56	139.66
		2h	10.48	10.20	74.70	72.09	172.90	160.13
		3h	12.00	11.30	80.93	78.6	183.73	176.24
	0.001	1h	15.33	15.70	100.05	97.61	225.76	220.31
		2h	25.67	28.10	205.18	200.7	430.11	410.26
		3h	16.50	16.72	110.20	108.31	220.40	208.32
	0.002	1h	13.98	13.80	82.63	80.91	180.62	175.36
		2h	14.30	14.50	92.65	90.32	200.36	203.79
		3h	13.67	13.70	82.30	80.12	178.50	175.31
	0.003	1h	11.35	11.40	70.98	71.03	150.75	145.88
		2h	11.00	11.11	67.60	67.5	145.82	140.28
		3h	10.03	10.20	66.50	64.7	148.82	141.13
	0.004	1h	10.05	10.05	56.11	56.50	123.48	120.82
		2h	10.00	9.76	55.23	53.13	128.11	119.76
		3h	8.05	8.00	45.50	46.40	101.97	96.35
Lincoln	Distilled water	1h	12.33	12.60	83.60	84.70	195.45	189.54
		2h	13.50	13.70	84.70	83.12	200.47	195.66
		3h	13.45	13.50	97.68	97.00	225.97	219.88
	0.001	1h	18.18	18.20	112.73	110.20	225.88	220.13
		2h	17.77	17.50	100.48	98.00	200.58	200.63
		3h	17.63	17.30	100.73	97.30	205.97	200.70
	0.002	1h	19.98	19.10	117.43	115.20	238.83	239.71
		2h	19.77	19.20	150.10	145.90	300.59	298.00
		3h	28.83	29.30	198.93	195.60	410.63	405.36
	0.003	1h	15.05	15.19	116.15	113.00	220.53	215.59
		2h	11.33	11.26	79.63	80.70	160.20	156.10
		3h	11.00	10.78	78.33	77.30	153.33	149.22
	0.004	1h	10.05	10.00	61.25	59.15	110.83	102.00
		2h	10.00	9.77	53.18	52.18	97.87	95.90
		3h	9.00	9.06	50.50	49.29	90.65	86.25
	L.S.D 0.5		1.12	1.13	4.47	4.03	3.75	3.50
	L.S.D 0.1		1.49	1.83	5.96	5.73	4.63	4.42

Concerning the cultivar Lincoln, the predictable dose was 0.002 for three hours that gave the highest significant values (28.83, 198,93 and 410.63 for number of pods per plant, number of seeds per plant, and yield of green pods per plant, respectively.

From (Table 6), it could be observed that the concentration "0.001" sodium azide for two hours gave the highest percent increase over Master B parent i.e. 144.94% 174.67% and 148.76% for number of pods per plant number of seeds and yield of green pods, respectively. Such low dose of the mutagen that gave the highest percent in yield and some of it's components may reflect the effect of the increase in catalase and peroxidase activities. On the other hand, 0.004 concentration for three hours gave sever reduction (29.58%) for number of pods per plant , (43.78%) for number of seeds per plant and 44.5% for yield of green pods per plant against the control. In M_1

derived from Lincoln the highest percent increase was under 0.002 sodium azide for three hours. This means that the increase in the mutagen dose form 0.001 for two hours to 0.002 for three hours, made such dose as a predictable dose to elevate and activate yield and some of it's components in Lincoln. This optimum dose gave 114.35%, 103.65% and 81.99% heterotic values for number of pods per plant, number of seeds and yield of green pods per plant, respectively.

Table (6) Elevation and depression % in yield and some of it's components as affected by sodium azide mutagen, in M_1 pea plants through two successive seasons (2003-2004)and (2004-2005).

Cultivars	Sodium azide concentr-ation	Time of soaking (hours)	N. of pods / plant		N. of seeds / plant		Yield of green pods / plant(g)	
			First season	Second season	First season	Second season	First season	Second season
Master B	0.001	1h	65.19	64.74	61.68	61.77	57.26	57.75
		2h	144.94	175.51	174.67	178.4	148.76	156.20
		2h	37.50	47.96	36.17	37.79	19.96	18.20
	0.002	1h	50.65	44.81	33.53	34.11	25.81	25.56
		2h	36.45	42.16	24.03	25.44	15.88	27.27
		3h	13.92	21.24	1.62	1.93	-2.85	0.53
	0.003	1h	22.31	19.83	14.71	17.74	5.01	4.45
		2h	4.96	8.92	-9.50	6.25	-15.66	12.40
		3h	-16.42	9.73	-17.83	17.68	-19.00	20.04
	0.004	1h	8.30	4.93	-9.32	5.85	-13.99	13.49
		2h	-4.58	4.31	-26.06	26.2	-25.91	25.21
		3h	-29.58	29.2	-43.78	40.93	-44.50	45.33
Lincoln	0.001	1h	47.45	44.44	34.84	30.11	15.57	16.14
		2h	31.63	27.73	18.63	17.90	0.05	25.29
		3h	31.08	28.22	3.12	0.31	-8.71	2.54
	0.002	1h	62.04	51.59	40.47	34.78	22.19	24.36
		2h	46.44	40.15	77.17	75.53	49.94	52.31
		3h	114.35	117.04	103.65	101.65	81.99	84.36
	0.003	1h	22.06	20.55	38.94	33.41	12.83	13.74
		2h	-16.07	17.8	-5.99	2.91	-20.14	20.92
		3h	-18.22	20.15	-19.81	20.31	-32.04	32.14
	0.004	1h	-18.49	20.63	-26.73	30.16	-43.29	46.12
		2h	-25.93	28.67	-37.21	37.22	-51.18	50.99
		3h	-33.09	32.89	-48.30	49.19	-59.82	60.77

The previous results agree with those reported, in different crops , by Reddy (1992), Mihov *et al* (2001) and Naglaa (2001) who mentioned that the concentration 0.25 of Ethyle imine decreased yield in tritical in additon of significant interaction between treatments of chemical mutagen and cultivars for yield and some of it's components. Moreover, Azza (2004) in faba bean used same concentrations of sodium azide and obtained superior mutants in yield and some related characters.

B- The second season (2004-2005) recorded the same trend of result.

3-Biochemical studies

(a) Electrophoretic studies:-

Electrophoresis banding patterns (SDS-PAGE) of extracted protein from dry seeds of two pea cultivars, Master B and Lincoln and their Mi generation are presented in figure (I and 2)

The cultivars:-

Four major regions are detected "R_1, R_2, R_3 and R_4" for both two cultivars, Master B and Lincoln. (Fig 1) R_1 region containes three major bands, two out of them are dark and the other is faint.(R_2) region consists of one major band. Such major band is distinguished with the increase of its density in Master B more than Lincoln cultivar. Three major bands were observed in the third region (R_3).As for R_4 region which indicated three bands, the two cultivars also were different.

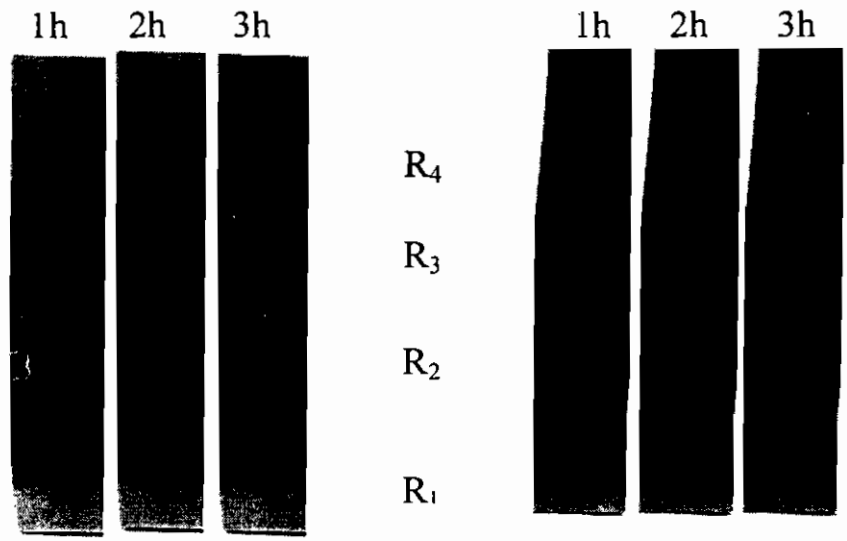

Fig (1): SDS-PAGE Profiles of seed protein according to their density and intensity for the two cultivars, Master B and Lincoln soaked with distilled water.

From the previous conclusion, it could be noticed that, large differences were observed for the major protein banding patterns of the two cultivars, Master B and Lincoln. Such differences in size and density make one assumes that the variation in banding patterns are genotypically and evolutionary different. This was substantiated by the facts that some of the subtractions of a particular protein were either slightly disappeared or were reduced in size and mobility. Such quantitative variations in the two cultivars banding patterns could be found if one assumes that the genes responsible for these metabolic phenomena are different in their action. A reasonable explanation that could be forward, is that these cultivars are of different origins and they have gone through completely different paths during evolutionary processes. Same results were obtained by Ismail and El-Ghareeb in cowpea (2000) and El-Ghareeb *et al.*, (2004) in tomato.

M_1 generation:

SDS-PAGE profiles of seed protein banding patterns according to their density and intensity for M_1 generation derived from soaking pea

cultivars with different sodium azide levels are presented in Fig (2). Great appearance is observed in M_1 generation, either in size of protein banding patterns or intensity as well as the increase in band number. All M_1,s were characterized by increasing band number and intensity in spite of having the same number of regions. "four"

Comparing the major bands of the M_1, s with their respective cultivars it could be concluded that .Region (1) that had slightly dark appearance in the two cultivars represented as a control, showed an increase in density in their (M_1.s). The appearance of the other three major regions showed also very dark and high intensities as well as some faint bands that were absent in the control cultivars before mutagen soaking. All M_1.s recorded dark stained bands "heavy molecular weight". Such intensities have unequal distribution and appearance in the two cultivars, soaked under sodium azide concentrations.

According to the effect of mutagen treatment, M_1 of Master B showed the highest appearance in density and intensity and more than M_1 of Lincoln. It could be clear that the treatment "0.001" of sodium azide gave the highest band density and intensity. The de novo appearance of these darkly stained "heavy molecular weight " bands in the M_1s, reflected over dominance action frame for the genes that control a particular protein fraction. These results were very close to those obtained by Amet (1992) who detected not only quantitative variability, presence / absence of bands but also qualitative variability in band number and intensities among the genotypes.

Fig. (2) Shows that the appearance of very distinctive darkly stained protein bands and increase in band number are identified as the effect of the low concentration of sodium azide mutagen. These results look like the increase in band number and intensity in F_1s and are very near with those obtained by normal hybridization methods. Thus there is no time waste moreover efforts can be saved. Ismial and El-Ghareeb(2000) reported same results in F_1s derived from crossing in cowpea. Moreover El-Ghareeb *et al* (2004) in tomato reported same results from heterotic performance on some electrophoretic protein banding patterns in tomato with relation to yield and some of it's components. They mentioned that the best F_1,s not only had the highest values in yield and it's components but also distinguished with the highest band number and intensities.

B- Protein content

Protein content % and their standard errors, elevation % as well as depression % for M_1 of both Master B and Lincoln as affected by sodium azide levels are shown in table (7). It was clearly observed that in M_1 of Master B, 0.001 concentration for two hours recorded an increase in seed protein at frequency (16.73%). On the other hand the concentration 0.002 for three hours gave (13.07%) for the M_1 of Lincoln. Meng *et al* (2002) obtained significant variations in protein in common bean.

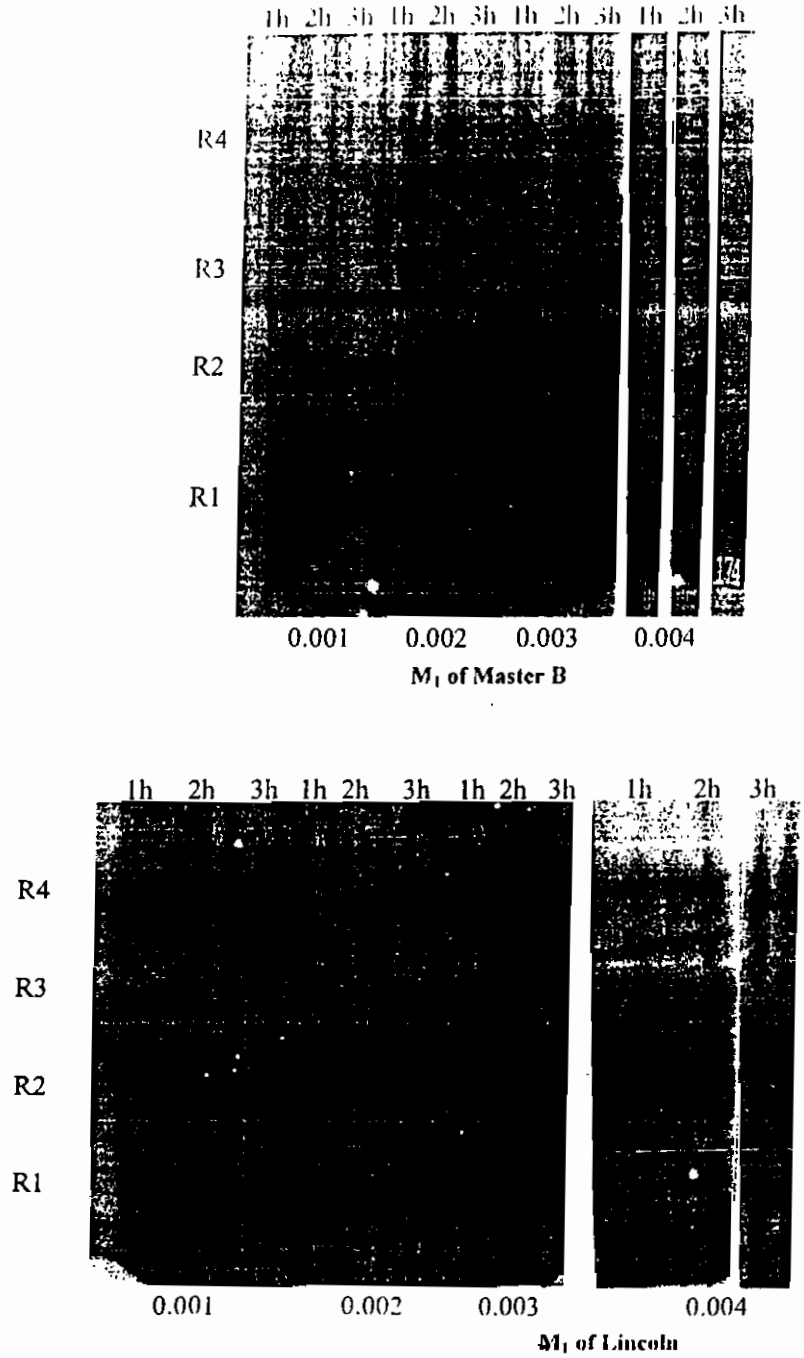

Fig (2): SDS- PAGE profiles of seed protein banding patterns according to their density and intensity for M_1 generation of Master B and Lincoln under different concentrations of sodium azide mutagen for three times of soaking

Table (7): Protein content % with their standard errors, elevation and depression % for M_1 generation of Master B and Lincoln under different sodium azide concentrations.

Treatments	Time of soaking (hours)	Master B		Lincoln	
		Protein content ± S.E	Elevation or depression%	Protein content ± S.E	Elevation or depression%
Distilled water	1h	18.00 ± 0.01		18.7 ± 0.15	
	2h	18.05 ± 0.03		18.9 ± 0.26	
	3h	18.18 ± 0.05		18.67 ± 0.20	
0.001	1h	20.00 ± 0.30	11.11	19.63 ± 0.17	4.97
	2h	21.07 ± 0.19	16.73	19.76 ± 0.15	4.55
	3h	19.63 ± 0.20	7.98	19.83 ± 0.18	6.21
0.002	1h	19.30 ± 0.12	7.22	10.05 ± 0.18	7.22
	2h	19.50 ± 0.15	8.03	20.13 ± 0.27	6.51
	3h	19.7 ± 0.18	8.36	21.11 ± 0.16	13.07
0.003	1h	18.12 ± 0.15	0.67	18.13 ± 0.11	- 3.05
	2h	18.13 ± 0.17	0.44	18.22 ± 0.08	- 3.60
	3h	18.09 ± 0.12	- 0.5	18.35 ± 0.13	- 1.71
0.004	1h	17.91 ± 0.09	- 0.5	18.03 ± 0.11	- 3.58
	2h	18.02 ± 0.07	- 0.17	18.12 ± 0.09	- 4.27
	3h	18.1 ± 0.09	- 0.44	18.07 ± 0.14	- 3.21

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تأثر المطفر الكيميائي في تحسين بعض الصفات المحصولية والبيوكيميائية في البسلة

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في موسمين متتالين (٢٠٠٣-٢٠٠٤) ، (٢٠٠٤-٢٠٠٥) تم نقع بذور الصنفين ماستر بي ولنكولن في الصوديوم أزيد بتركيزات ٠,٠٠١ ، ٠,٠٠٢، ٠,٠٠٣ ، ٠,٠٠٤ لمدة ساعة وساعتين وثلاثة ساعات واستخدام تصميم القطع تحت المنشقة في أربعة مكررات وكانت النتائج كالتالي:-
في الموسم الاول (٢٠٠٣-٢٠٠٤).

١-**النمو الخضري:**- أعطي التركيز ٠,٠٠٢ لمدة ساعتين من المطفر الكيماوي الصوديوم أزيد اعلي القيم العالية معنوية (٩٣,٧٥% ، ٦٨,٣٨سم ، ٢١,٥٥ ، ٣,٢٦) بزيادة قدرها ١٥,٨٨% ، ٨٢,٦٩ % ، ٥٧,٠٧% ، ١٠٠,٨٧% عن الأب ماستر بي في الجيل الطفري الأول وذلك للصفات نسبة الإنبات، ارتفاع النبات ، عدد الأوراق وعدد الفروع للنبات الواحد علي الترتيب بينما أعطت الجرعة ٠,٠٠٤ لمدة ساعتين نقصا ملحوظا (٢١,٨٧% ، ٢٢,٥٨%) لعدد الأوراق والفروع وأعطت نفس الجرعة ولكن لمدة ٣ ساعات نقصا ١٧,٥٢% لنسبة الإنبات ، ١٤,٠١% لارتفاع النبات وفي الجيل الطفري الأول للصنف لنكولن أعطي التركيز ٠,٠٠٢ أيضا لمدة ساعتين اعلي قيم معنوية لنسبة الإنبات (٩٤,٧٨%) ، (٩٨,١٣سم) لارتفاع النبات و ٤,٨٨ لعدد الفروع وحينما زادت مدة نقع البذور إلي ٣ ساعات أعطي نفس التركيز اعلي قيمة (٢٤,٤٠) لعدد الأوراق. وذلك بزيادة عن الأب لنكولن مقدارها (١٢,٣٩% ، ٥٠,٢١%، ٦٤,٥٣%، ٩٢,٨٩%) لنسبة الإنبات وارتفاع النبات وعدد الأوراق والفروع للنبات الواحد وكانت نسبة النقص عند التركيز ٠,٠٠٤ لمدة ٣ ساعات (١٧,٤٨% ، ٢٣,٩١% ، ٢٤,٤٨% ، ٦٣,٢١%) للصفات الخضرية نفسها علي الترتيب.

٢- **المحصول ومكوناته:**- في الجيل الطفري الأول للصنف ماستر بي أعطي التركيز ٠,٠٠١ صوديوم أزيد لمدة ساعتين اعلي القيم عالية المعنوية لعدد القرون (٢٥,٦٧)، (٢٠٥,١٨) لعدد بذور النبات، (٤٣٠,١١جم) لمحصول القرون الخضراء. وذلك بزيادة قدرها(١٤٤,٩٤% ، ١٧٤,٦٧% ، ١٤٨,٧٦%)للصفات نفسها علي الترتيب وأعطت الجرعة ٠,٠٠٤ ولمدة ٣ ساعات عكس النتائج السابقة.

إما في الجيل الطفري الأول للصف لنكولن كان التركيز المثالي للمطفر الصوديوم أزيد ٠,٠٠٢ لمدة ٣ ساعات حيث أعطت اعلي القيم المعنوية (٢٨,٨٣ ، ١٩٨,٩٣ ، ٤١٠,٦٣جم) لعدد القرون وعدد بذور النبات ومحصول القرون الخضراء علي الترتيب بزيادة قدرها (١١٤,٣٥% ، ١٠٣,٦٥% ، ٨١,٩٩%) عن الأب لنكولن. كما أعطت الجرعة ٠,٠٠٤ نقصا ملحوظا في عدد القرون والبذور والمحصول الكلي للقرون الخضراء بمعدل (٣٣,٠٩% ، ٤٨,٣% ، ٥٩,٨٢%)

ب- في الموسم الثاني: (٢٠٠٤-٢٠٠٥) تم الحصول علي نفس النتائج تقريبا.

٣- الدراسات البيوكيميائية:

أ- إن أعلي عدد من حزم البروتين وأعلي كثافة قد تم الحصول عليها من التركيزات ٠,٠٠١ ، ٠,٠٠٢ صوديوم أزيد مقارنة بباقي المعاملات وكذلك الآباء وأن هذه النتيجة تشابه وقريبة من التي يمكن الحصول عليها بطريقة التهجين.

ب- أظهرت النتائج أن أعلي نسبة زيادة في محتوي البروتين كانت ١٦,٧٣% من التركيز ٠,٠٠١ صوديوم أزيد لمدة ساعتين للجيل الطفري الأول للصنف ماستر بي بينما تركيز ٠,٠٠٢ لمدة ٣ ساعات أعطت زيادة ١٣,٠٧ % في الجيل الطفري الأول للصنف لنكولن.