Egypt. J. Plant Breed. 12(1): 41-56 (2008)

EFFECT OF SODIUM AZIDE AND COLCHICINE ON ZEBDA AND COMPANY MANGOES

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ABSTRACT

This experiment was carried out during 2004/2005 and 2005/2006 seasons to study the effect of two chemical mutagens (sodium azide and colchicine) of the Egyptian varieties Zabda and Company polyembryonic mango seeds. Drench application at 0.8% with spraying at 0.008 ppm sodium azide was very effective in reducing plant height and increasing stem diameter than the control in both varieties. All treatments reduced number of leaves in both cultivars and seasons compared with control. Meanwhile, spraying sodium azide at 0.008 ppm for Zebda and 0.004 ppm for Company resulted in the largest leaf area. Spraying sodium azide at 0.002 ppm on the nuceller seedling of Zebda variety led to increase chlorophyll A and carotenoids in leaves, but spraying colchicine at 0.004 ppm increased chlorophyll B in leaves. Leaf content of chlorophyll A and B and caroten of Company cultivar increased by using sodium azide at 0.2% drench plus sprayed at 0.002 ppm. Drench application of sodium azide at 0.8% plus spraying it at 0.008 ppm led to increase in total indoles but spraying sodium azide at 0.008 ppm caused an increase in total phenols in Zebda leaves.Using both sodium azide and colchicine reduced total phenols, but sprayed it only at 0.008 ppm caused an increase in total indoles in Company leaves. At the molecular level, there was no significant differences between the Zebda and Company varieties in the appearance of new bands when sodium azaide was used with any of the tested concentrations. On the other hand Company variety was more affected by colchicine at different levels either in the absence or presense of new bands. Moreover, genetic similarity showed a wide variation in the distances either among the different concentrations or the tested varieties.

Key words: ***Mango, Mutagens, Sodium azide, Colchicine, (RAP), Chemical composition.***

INTRODUCTION

Mango, *Mangifera indica* L., belongs to the family *Anacardiceae*. Its tree is evergreen, native to South Eastern Asia, from India to the Philippine (Purseglove 1972). Egypt is considered one of the major mango producing countries in Africa. It produces a huge tonnage of mango fruits. However, a small percentage of the production is primarily exported to regional markets (Litz 1997).

Great variations and differences can be noticed in vegetative growth between the trees of the same variety and at the same age grown in one orchard of mango. This variation could be due to the rootstock or soil conditions. The use of mutagens in crop improvement helps to understand the mechanism of mutation induction and to quantify the frequency as well as the pattern of changes in induced mutants. Mutation breeding generates a knowledge base that guides future users of mutation technology for crop

improvement. The mutagenic effects of sodium azide have been documented. Youssef (2003) on *Yucca elephamtipes* medicus *and philodendron scandens* L. reported that sodium azide and colchicine induced chlorophyll deficiency as well as a wide range of morphological and physiological mutants.

On the other hand, colchicine is both polyploidizing and mutagenic agent (Bragal 1955). This chemical has been used for a long time to produce polyploid plants. The mutagenic effects on plant morphology, chlorophyll, sterility and yield have earlier been confirmed by Mensah (1977), and Castro *et al* (2003). Balkanjieva (1980) has reported the role of genotype on the induced mutagenic variability in barley following colchicine treatment.

MATERIALS AND METHODS

The present work was conducted for two successive seasons (2004/2005 and 2005/2006) to study the effect of some chemical mutagens induced dwarfing on genetic variation of some mango varieties. During August 2003, seeds of two polyembryonic mango varieties, namly Zebda and Company were extracted from the fruits, cleaned, washed using tap water and dried at room temperature, then seeds were sown at rows in seed bed in the shade greenhouse of the Horticulture Research Institute, Giza, Egypt. After 2-3 weeks of seed germination, when the seedlings began to turn from purple to green colour, seedlings were transplanted to black plastic bags (20×35 cm) filled with a soil mixture consisted of fine sand, compost and silty soil at 3:1:1 in volume, respectively.

The nuceller seedlings produced from the Zebda and Company polyembryonic seeds (3-4 seedlings for each) were separated and transplanted individually as previously mentioned, and arranged in the greenhouse shade, while the sexual seedlings were discarded.

Zebda and Company nuceller seedlings were subjected to 13 treatments for each variety, each treatment included 3 seedlings plants in 3 replicates.

The treatments were as follows

1- Control (untreated).
2- Sodium azide drench at 0.2, 0.4 and 0.8% in 1st and 31st of December and spray at (0.002, 0.004 and 0.008 ppm) respectively (in 1st of April and the1st of May).
3- Sodium azide spray at 0.002, 0.004 and 0.008 ppm(in the1st of April and the 1st 0f May).
4- Colchicine drench at 0.2, 0.4 and 0.8 % (in the1st and 31st of December) and spray at 0.002 and 0.008 ppm respectively (in the1st of April and the1st of May).

5- Colchicine spray at 0.002, 0.004 and 0.008 ppm (in the 1st of April and the1st of May).

Data recorded

The vegetative growth

Plant height, stem diameter, number of leaves, and leaf area characters were recorded after 6 month in1st of December.

Chemical compositions

Chlorophyll A and B and carotenoids in mg/g were determined in leaves of plants according to Saric *et al* (1967).
Total indoles were determined according to Selim *et al* (1978).
Total phenolic compounds were determined according to Daniel and George (1972).

DNA extraction and RAPD analysis:

Total DNA was extracted from young leaves using hexdecy 1-trimethyl-ammonium bromide (CTAB) procedure based on the protocol of Porebski *et al* (1997). Ten RAPD primers (Table 1) were used for PCR amplification. Each 25µl amplification reaction consisted of 10 µl Tris-HCl, 2 mM $MgCl_2$, 200 µM each dNTPS, 1 µM primers, 1 unit of Taq, DNA polymerase and 25ng of template DNA.

Table 1. The ten RAPD primers used in this study

Primer	Primer sequence
OPA01	CAGGCCCTTC
OPA08	GTGACGTAGG
OPC02	GTGAGGCGTC
OPC10	TGTCTGGGTG
OPC15	GACGGATCAG
OPO03	CTGTTGCTAC
OPO04	AAGTCCGCTC
OPO20	ACACACGCTG
OPZ15	CAGGGCTTTC
OPZ20	ACTTTGGCGG

Amplification was performed in a thermal cycler using the following temperature profile: 4 min. at 94°C, followed by 40 cycles each cycle consisted of adenaturation step at 94°C for 1 min., an annealing step at 37°C for 2 min and an elongation step at 72°C for 2 min. The primer extension segment was extended to 7 min at 72°C in the final cycle. The amplification products were resolved by electrophoresis in 1.5% agarose gel in 1× TBE buffer at 95 volts for two hrs.

Data analysis

The experiment was set in two factor Randomized Complete Block Design and data were subjected to statistical analysis according to Sendecor and Cochran (1980).

Polymorphic fragments were scored as present (scored as 1) or absent (scored as 0). A similarity matrix with similarity coefficients of Dice (1995) was performed based on the presence or absence of polymorphic fragments for each primer. The Dice coefficient is identical to that of Nei and Li (1979).

RESULTS AND DISCUSSION

Vegetative growth

Plant height

Data presented in Table (2) show the effect of sodium azide and colchicine treatments on height of nucellar seedlings of Zebda and Company mango varieties.

These results indicated that height of seedling significantly decreased with using of both chemical mutagens as compared to control in both cultivars and seasons. The reduction was significantly correlated with the increment in both chemical mutagen concentrations. It can be seen that chemical mutagens at 0.008 ppm concentration caused a significant reduction in plant height.

In addition, using sodium azide induced more decreased in plant height of both varieties (Zebda and Company) as compared with colchicine.

Meanwhile, it is clear from Table (2) that treating plants with both drench and spray caused significantly more reduction in plant height of Zebda and Company varieties in both chemical mutagens than using spray treatment only.

With respect of the effect of interaction between chemical mutagen concentration and method of application, data in Table (2) showed that sodium azide at 0.008 ppm treated with both drench and spray together as a method of application gave the highest reduction in plant height (41.33 and 39.00 cm) for Zebda and (49.67 and 43.00 cm) for Company in both seasons,respectively.

Table 2. Effect of different mutagen treatments and methods of application on plant height and diameter of the nuceller seedlings of Zebda and Company mango cultivars in 2004/2005 and 2005/2006 seasons.

Zebda												
	Plant height (cm.)						Stem diameter (cm.)					
	2004/2005			2005/2006			2004/2005			2005/2006		
Treatment	Spray	Drench	Mean	Spray	Drench	Mean	Spray	Drench	Mean	Spray	Drench	Mean
Control	44.16	44.16	44.16	47.17	47.17	47.17	0.54	0.54	0.54	0.60	0.60	0.60
Naz.002	41.67	43.00	42.34	45.67	43.00	44.34	0.70	0.77	0.74	0.70	0.63	0.67
Naz.004	41.67	41.67	41.67	44.33	40.33	42.33	0.50	0.75	0.63	0.63	0.60	0.62
Naz.008	41.33	41.33	41.33	44.33	39.00	41.67	0.46	0.60	0.53	0.63	0.93	0.78
Col.002	43.67	42.33	43.00	44.33	43.67	44.00	0.56	0.73	0.65	0.67	0.87	0.77
Col.004	42.33	42.00	42.17	44.05	-	22.03	0.50	0.80	0.80	0.65	0.10	0.38
Col.008	-	-	-	-	-	-	-	-	-	-	-	-
Mean	36.40	36.36		38.55	30.45		0.46	0.60		0.55	0.53	
L.S.D Method		N.S			1.39			0.08			0.02	
L.S.D. Treatment		1.67			2.59			0.16			0.06	
L.S.D interaction		2.37			3.67			0.22			0.09	
Company												
Control	59.33	59.33	59.33	62.67	62.67	62.67	0.53	0.53	0.53	0.57	0.57	0.57
Naz.002	58.00	51.00	54.50	60.00	55.33	57.67	0.56	0.57	0.57	0.67	0.80	0.74
Naz.004	59.50	50.50	55.00	62.00	48.50	55.25	0.46	0.65	0.56	0.57	0.85	0.71
Naz.008	62.33	49.67	56.00	62.33	43.00	52.67	0.46	0.90	0.68	0.57	0.85	0.71
Col.002	57.00	50.00	53.50	60.00	52.33	56.17	0.43	0.77	0.60	0.57	0.70	0.64
Col.004	57.00	56.50	56.75	62.25	55.00	58.63	0.43	0.87	0.65	0.55	0.73	0.64
Col.008	-	59.00	29.50	-	55.50	27.75	-	0.87	0.44	-	0.73	0.37
Mean	50.45	52.90		52.75	53.19		0.41	0.74		0.50	0.75	
L.S.D Method		0.85			N.S			0.07			0.08	
L.S.D. Treatment		4.76			4.44			0.14			0.16	
L.S.D interaction		6.74			6.28			0.20			0.22	

Naz=Sodium azide Col=Colchicine NS=non significant Missing data(dead plant) =

Stem diameter

It was obvious from Table (2) that in general, using both chemical mutagens increased stem diameter as compared to control in both cultivars and seasons.It was clear from Table (2) that stem diameter was significantly increased with sodium azide as compared with colchicine.

As for Zebda variety using sodium azide at 0.008 ppm and colchicine at 0.002 ppm resulted in the maximum stem diameter .Meanwhile, using sodium azide at 0.008 ppm in the first season and at 0.002 ppm in the second season caused the highest significant increase in stem diameter for Company variety.

Concerning the method of application, data in Table(2) showed that, in the first and second seasons using both drench and spray together as a method of application caused the highest increase in stem diameter in the two mango varieties.

From Table (2) it can be observed that treating with drench and spray together with sodium azide at 0.008 ppm resulted in the maximum stem diameter in the first and second seasons with the two mango cultivars. While spraying only with sodium azide at 0.002 ppm resulted in the highest significant increase in stem diameter at the second season in both Zebda and Company varieties.

Leaf number

Data in Table (3) showed the effect of different concentrations, method of application with sodium azide and colchicine on leaf number of Zebda and Company mango varieties. It was clear that using of both chemical mutagens reduced leaf number in both varieties and both seasons as compared with control.

Concerning the effect of concentration , the reduction in leaf number was significantly correlated with the increasing of concentration for the two chemical mutagens. These results are true in both cultivars and both seasons.

On the other hand, using sodium azide at 0.002 ppm resulted in the highest leaf number in both cultivars and both seasons as compared with the other concentrations of sodium azide and all concentrations of colchicine.

As for the method of application, spraying the nucellar seedlings of Company variety with sodium azide or colchicine caused more increase in leaf number than both drench and spray together in the two studied seasons.

Meanwhile, in Zebda nucellar seedlings using both drench and spray treatments together in the first season caused a significant higher increase in leaf number than using spray only, the second season gave an opposite result.

Table 3. Effect of different mutagen treatments and methods of application on leaf number and area of the nuceller seedlings of Zebda and Company mango cultivars in 2004/2005 and 2005/2006 seasons.

Zebda												
	Leaf number						Leaf area (cm^2)					
	2004/2005			2005/2006			2004/2005			2005/2006		
Treatment	**Spray**	**Drench**	**Mean**	**Spray**	**Drench**	**Mean**	**Spray**	**Drench**	**Mean**	**Spray**	**Drench**	**Mean**
Control	27.67	27.67	**27.67**	20.67	20.67	**20.67**	83.84	83.84	**83.84**	67.50	67.50	**67.50**
Naz.002	19.33	25.00	**22.17**	19.67	17.67	**18.67**	79.83	81.00	**80.42**	79.55	56.73	**68.14**
Naz.004	14.33	17.33	**15.83**	18.33	15.17	**16.75**	90.08	83.33	**86.71**	104.00	66.65	**85.33**
Naz.008	14.33	21.33	**17.83**	16.33	16.17	**16.25**	93.25	95.00	**94.13**	107.60	85.50	**96.55**
Col.002	16.67	24.00	**20.34**	18.00	19.67	**18.84**	80.00	80.25	**80.13**	77.67	100.00	**88.84**
Col.004	16.67	-	**8.34**	15.00	-	**15.00**	90.75	92.67	**91.71**	120.00	-	**60.00**
Col.008	-	-	-	-	-	**0.00**	-	-	-	-	-	-
Mean	**15.57**	**16.48**		**15.43**	**14.89**		**73.96**	**73.73**		**79.47**	**53.77**	
L.S.D Method		**N.S**			**N.S**			**N.S**			**0.77**	
L.S.D. Treatment		**4.30**			**3.13**			**2.07**			**1.43**	
L.S.D interaction		**6.08**			**4.42**			**2.92**			**2.03**	
TREAT	**Company**											
Control	18.50	18.50	**18.50**	17.67	17.67	**17.67**	86.79	86.79	**86.79**	124.30	124.30	**124.30**
Naz.002	18.00	15.00	**16.50**	17.00	12.33	**14.67**	104.50	76.33	**90.42**	157.40	156.60	**157.00**
Naz.004	17.00	9.67	**13.33**	16.33	10.00	**13.17**	126.80	104.50	**115.65**	265.50	166.40	**215.95**
Naz.008	17.33	10.00	**13.67**	14.00	7.00	**10.50**	120.00	76.25	**98.13**	220.00	95.00	**157.50**
Col.002	17.33	13.33	**15.33**	12.67	15.00	**13.84**	88.25	139.40	**113.83**	126.80	144.20	**135.50**
Col.004	17.33	12.67	**15.00**	13.67	14.00	**13.84**	90.75	122.10	**106.43**	157.50	126.00	**141.75**
Col.008	-	12.33	**6.17**	-	7.00	**3.50**	-	126.00	**63.00**	-	176.30	**88.15**
Mean	**15.07**	**13.07**		**13.05**	**11.86**		**88.16**	**104.48**		**150.21**	**141.26**	
L.S.D Method		**1.16**			**N.S**			**0.79**			**0.76**	
L.S.D. Treatment		**2.17**			**2.49**			**1.48**			**1.42**	
L.S.D interaction		**3.06**			**3.52**			**2.09**			**2.00**	

Naz=Sodium azide **Col=Colchicine** **NS=non significant** **Missing data(dead plant) = -**

Regarding the interaction between mutagen concentration and method of application, data in Table (3) showed that using colchicine at 0.004 ppm for drench and spray together with Zebda variety in the first season resulted in the highest leaf number after control. But in the second season, spraying Zebda nucellar seedlings with 0.002 ppm sodium azide gave the best results after control. On the other hand, spraying Company nucellar seedlings by sodium azide at 0.002 ppm resulted in the highest leaf number after control as compared with other interactions in the two seasons.

Leaf area

Data in Table (3) showed the effect of sodium azide and colchicine concentrations on leaf area of nucellar seedlings of Zebda and Company mango varieties. It was clear that sodium azide induced more increase in leaf area as compared with control and as compared with colchicine in the two varieties and two seasons.

Using sodium azide at 0.008 ppm with Zebda variety and at 0.004 ppm with Company cultivar gave the maximum leaf area in the two seasons.

Regarding the effect of method application, Table (3) showed that spraying Zebda nucellar seedlings resulted with largest leaf area in the two seasons as compared with treating with drench and spray together.

Meanwhile, in Company nucellar seedlings treating with drench and spray together gave the largest leaf area as compared with spray only in the second season. Colchicine induced higher polyploidy, stickness, aneuploidy and micronucleated cells as well as medium chromosomal fragments, Meanwhile sodium azide encouraged higher polyploidy, micronucleated cells, and stickness Youssef (2003) on *Yucca elephamtipes* medicus *andphilodendron scandens* .L.

Chemical composition

Chlorophyll A and B and carotenoids

Chlorophyll, which is the green pigment in leaves, is very important in plant life through the process of photosynthesis. The amount of chlorophyll produced per gram leaf tissue is affected by environmental conditions and genetic composition of the plant.

Results in Table (4) showed that, sodium azide induced a significant increase in chlorophyll A and B and in carotenoids as compared with colchicine in both varieties.

Table 4. Effect of different mutagen treatments and methods of application on chlorophyll A andB and carotenoids of the nuceller seedlings of Zebda and Company mango varieties in 2005/2006 season.

Zebda									
	Chlorophyll" A"mg/g			Chlorophyll" B"mg/g			Carotenoids mg/g		
Treatment	Spray	Drench	Mean	Spray	Drench	Mean	Spray	Drench	Mean
Control	1.27	1.27	**1.27**	0.43	0.43	**0.43**	0.94	0.94	**0.94**
Naz.002	3.07	1.75	**2.41**	0.66	0.55	**0.61**	2.79	1.57	**2.18**
Naz.004	2.39	2.81	**2.60**	0.79	0.62	**0.71**	2.02	2.52	**2.27**
Naz.008	1.91	2.42	**2.17**	0.62	0.80	**0.71**	1.62	2.23	**1.93**
Col.002	2.07	1.21	**1.64**	0.84	0.35	**0.60**	1.81	1.10	**1.46**
Col.004	1.01	-	**0.51**	0.59	-	**0.30**	1.05	-	**0.53**
Col.008	-	-	**0.00**	-	-	-	-	-	**0.00**
Mean	**1.67**	**1.35**		**0.56**	**0.39**		**1.46**	**1.19**	
LSD Method		**0.04**			**N.S**			**0.01**	
LSD treatment		**0.08**			**0.35**			**0.02**	
LSD interaction		**0.11**			**0.49**			**0.03**	
Company									
Control	1.67	1.67	**1.67**	0.26	0.26	**0.26**	1.67	1.67	**1.67**
Naz.002	1.53	4.70	**3.12**	0.51	1.53	**1.02**	1.13	4.08	**2.61**
Naz.004	1.91	2.60	**2.26**	1.26	1.05	**1.16**	1.22	2.01	**1.62**
Naz.008	2.66	2.96	**2.81**	0.86	0.93	**0.90**	2.20	2.58	**2.39**
Col.002	1.94	2.06	**2.00**	0.60	0.99	**0.80**	1.67	1.62	**1.65**
Col.004	1.79	3.13	**2.46**	0.56	0.87	**0.72**	1.48	2.72	**2.10**
Col.008	-	2.28	**1.14**	-	0.72	**0.36**	-	2.03	**1.02**
Mean	**1.64**	**2.77**		**0.58**	**0.91**		**1.34**	**2.39**	
LSD Method		**0.03**			**0.07**			**0.27**	
LSD treatment		**0.05**			**0.14**			**0.50**	
LSD interaction		**0.08**			**0.19**			**0.71**	

Naz=Sodium azide Col=Colchicine Missing data(dead plant) = -

As for mutagen concentrations, data revealed that sodium azide at 0.004 ppm gave the significant increase in chlorophyll A and at 0.008 ppm in chlorophyll B and carotenoids for Zebda variety. Meanwhile in Company variety, sodium azide at 0.002 ppm gave the significant increase in chlorophyll A and B and in carotenoids.

Concerning the effect of method application, data in Table (4) showed that treating with spray only produced the significant increase in chlorophyll A and B and carotenoids with Zebda variety.

On the other hand treating drench and spray together of Company nucellar seedlings produced the significant increase in chlorophyll A and B and carotenoids. Results showed that spraying sodium azide at 0.002 ppm gave the highest chlorophyll A and carotenoids contents in Zebda variety. Meanwhile, spraying colchicine at 0.004 ppm gave the highest chlorophyll B content as compered with other treatments in Zebda variety.

As for Company nucellar seedlings, it was noticed that treatments of drench and spray together with sodium azide at 0.002 ppm gave the highest contents of chlorophyll A and B as will as carotenoids.

Total indoles and phenols

Data in Table (5) showed that, using sodium azide at 0.008 ppm induced the maximum content of total indoles and phenols in Zebda variety.

Regarding the method of application, spraying plants of Zebda gave the highest content of total indoles and phenols. As for interaction, using sodium azide at 0.008 ppm as drench and spray treatment together gave the maximum content of total indoles.

Meanwhile, spraying plants of Zebda variety with sodium azide at 0.004 ppm gave the maximum content of total phenols.

On the other hand, results in Table (5) showed that using sodium azide and colchicine on plants of Company variety reduced contents of total indoles and total phenols as compared with control.

While using 0.008 ppm sodium azide produced the lowest increase in the total indoles and phenols as compared with control.

Concerning the method of application, data showed that treatment with both drench and spray together produced the highest contents of indoles and phenols as compared with spray only.

As for combinations between treatments, spraying sodium azide at 0.008 ppm produced the lowest increase in total indoles as compared with control. On the other side, using sodium azide as drench and spray together at 0.008 ppm gave the highest increase in total phenols with Company variety.

Table 5. Effect of different mutagen treatments and methods of application on indoles and phenols of the nuceller seedlings of Zebda and Company mango varieties in 2005/2006 season .

	Zebda					
	Indoles			Phenols		
Treatment	Spray	Drench	Mean	Spray	Drench	Mean
Control	7.65	7.65	**7.65**	16.91	16.91	**16.91**
Naz.002	10.12	9.76	**9.94**	13.10	12.68	**12.89**
Naz.004	9.02	12.69	**10.86**	13.29	18.16	**15.73**
Naz.008	8.63	13.34	**10.99**	20.28	16.33	**18.31**
Col.002	8.14	8.33	**8.24**	13.97	15.82	**14.90**
Col.004	8.79	-	**4.40**	24.16	-	**12.08**
Col.008	-	-	-	-	-	**0.00**
Mean	**7.48**	**7.40**		**14.53**	**11.41**	
LSD method		**N.S**			**0.29**	
LSD treatment		**0.67**			**0.55**	
LSDinteraction		**0.95**			**0.77**	
	Company					
Control	14.33	14.33	**14.33**	20.42	20.42	**20.42**
Naz.002	10.04	3.90	**6.97**	13.71	21.58	**17.65**
Naz.004	10.50	12.15	**11.33**	12.16	15.81	**13.99**
Naz.008	12.84	10.84	**11.84**	16.16	24.63	**20.40**
Col.002	0.77	9.79	**5.28**	11.77	11.92	**11.85**
Col.004	8.77	11.35	**10.06**	16.73	8.96	**12.85**
Col.008	-	12.61	**6.31**	-	16.60	**8.30**
Mean	**8.18**	**10.71**		**12.99**	**17.13**	
LSD method		**0.22**			**N.S**	
LSD treatment		**0.41**			**11.38**	
LSDinteraction		**0.58**			**16.09**	

Naz=Sodium azide Col=Colchicine Missing data(dead plant) = -

DNA – RAPD analysis

In the present study, genetic changes resulted from using the chemical mutagens sodium azide and colchicine with the two varieties of mango Zebda and Company were summarized in Table (6). New DNA bands appeared, while others disappeared as a result of treatments.

In Company variety, the highest number (4) of new bands were recorded with OPCO2 at the concentration 0.002 ppm of colchicine. These new bands had a molecular weight of 1000, 650, 350 and 300 bp.

Moreover the highest number (2) of new bands was shown with Zebda variety at concentration 0.008 ppm of sodium azide with OPC15 with

Table 6. Summary of the new band (Nb), number of absent band (Ab) and conserved band (Cb) resulted from each treatment.

	OPA01			OPA08			OPC02			OPC10			OPC15			OPO03			OPO04			OPO20			OPZ15			OPZ20		
Zebda	**Nb**	**Ab**	**Cb**	**Nb**	**Ab**	**Cb**	**Nb**	**Ab**	**Cb**	**Nb**	**Ab**	**Cb**	**Nb**	**Ab**	**Cb**	**Nb**	**Ab**	**Cb**	**Nb**	**Ab**	**Cb**	**Nb**	**Ab**	**Cb**	**Nb**	**Ab**	**Cb**	**Nb**	**Ab**	**Cb**
Naz.002	-	-	18	1	1	13	-	-	18	-	-	10	-	-	14	-	-	17	1	-	8	1	1	11	1	1	14	1	-	11
Naz.004	-	1	18	-	1	14	-	-	18	-	-	10	1	-	13	-	-	17	1	-	8	-	1	12	1	1	14	1	-	11
Naz.008	-	1	18	1	1	13	1	-	17	-	-	10	2	2	12	-	-	17	1	-	8	1	1	11	2	-	12	1	-	11
Col.002	-	-	18	-	-	14	1	2	17	-	1	10	1	1	13	-	1	17	-	1	9	1	1	11	1	1	13	1	-	11
Col.004	-	-	18	-	1	14	1	1	17	-	-	10	-	1	14	-	-	17	-	-	9	-	1	12	1	1	13	1	-	11
Col.008	-	2	18	-	-	14	1	-	17	-	1	10	1	-	13	-	1	17	1	-	8	1	1	11	1	1	13	1	-	11
Total	0	0.3	108	2	4	32	4	3	104	0	2	60	5	4	79	0	2	102	4	1	50	4	6	68	7	5	79	6	0	66
Mean	0		18	0.3	0.6	13.6	0.6	0.5	17.3	0	0.3	10	0.8	0.6	13.2	0	0.3	17	0.66	0.36	8.33	0.66	1	11.33	1.6	0.83	13.16	1	0	11
Company	**Nb**	**Ab**	**Cb**	**Nb**	**Ab**	**Cb**	**Nb**	**Ab**	**Cb**	**Nb**	**Ab**	**Cb**	**Nb**	**Ab**	**Cb**	**Nb**	**Ab**	**Cb**	**Nb**	**Ab**	**Cb**	**Nb**	**Ab**	**Cb**	**Nb**	**Ab**	**Cb**	**Nb**	**Ab**	**Cb**
Naz.002	-	2	18	1	1	13	-	1	18	-	-	10	-	-	14	1	1	16	1	-	8	1	-	11	-	2	15	-	-	12
Naz.004	-	1	18	-	-	14	-	1	18	-	-	10	-	-	14	1	1	16	2	-	7	1	1	11	-	2	15	-	-	12
Naz.008	-	1	18	-	-	14	1	1	17	-	-	10	1	-	13	2	2	15	1	-	8	2	1	10	1	2	14	2	2	10
Col.002	-	1	18	-	-	14	4	2	14	-	-	10	1	-	13	2	2	15	-	-	9	2	1	10	1	2	14	1	2	11
Col.004	-	2	18	1	-	13	1	1	13	-	-	10	-	-	14	2	2	15	-	-	9	1	1	11	-	2	15	2	2	10
Col.008	-	3	18	-	2	14	1	-	13	1	1	9	1	-	13	1	2	16	1	-	8	2	1	10	1	2	14	2	2	10
Total	0	10	108	2	3	82	7	6	93	1	1	59	3	-	81	9	10	93	5	0	49	9	5	63	3	12	87	7	8	65
Mean	0	1.66	18	0.33	0.5	13.66	1.16	1	15.5	1.16	1.16	9.83	0.5	-	13.5	1.5	1.66	15.5	0.83	0	8.16	1.5	0.83	10.5	0.5	2	14.5	1.16	1.33	10.8

New band (Nb) **Absent band (Ab)** **Conserved band (Cb)** **Naz=Sodium azide** **Col=Colchicine**

Negative band = -

a molecular weight of 2500 and 1650 bp and OPZ 15 with a molecular weight of 1230 and 450 bp.

On the other hand, Zebda variety recorded the highest number of absent bands (2) with sodium azide 0.008 ppm by using the primers OPC15 ($_{2000}$) bp and OPC 15($_{1700}$) bp using and colchicine 0.002 ppm OPCo2 ($_{1850}$) bp and OPCo2 ($_{1450}$) bp.

However, the variety Company showed the highest number (3) of absent bands with colchicine using OPA01 ($_{2050}$) bp OPA01 ($_{750}$) bp and OPAo1 ($_{470}$) bp. It is clear that there is no significant differences between Zebda and Company in the appearance of new bands when sodium azide used with the tested concentrations. This may reflect that the two genotypes almost have the same sensitivity to sodium azide.

Company variety, showed differences in the total number of absent bands ranging from 7 to 10 for all used primers at concentrations of 0.002 and 0.008 ppm, respectively. However, the variety was more affected by colchicine using different concentrations either in the absence or presence of new bands than Zebda variety. Company variety showed new bands ranging from 7 to 11 with colchicine as compared with 4 to 6 for Zebda variety. The variety also recorded absent bands ranging from 9 to 13 as compared with 5 to 8 for Zebda cuttings (Table 6).

The scored data (1 for presence and 0 for absence) resulting from ten tested primers were used to compute the similarity matrices according to Dice (Sneath and Sokal 1973).

Table (7) reveals that the similarity percentage (85.6% and 81.3%) was high between the control and the first concentration of sodium azide (0.002 ppm) with the two mango genotypes Zebda and Company, respectively. The concentration 0.004 ppm of sodium azide decreased the similarity percent with the control in both of Zebda (80.1%) and Company (77.1%), while 0.008 ppm of sodium azide recorded similarity percent more higher than the second concentration (0.004 ppm) but less than the first one (0.002 ppm) in both Zebda and Company varieties .This may be due to type of DNA sequence variation detected (base substitution vs. insertion/deletion) and the type of sequence assayed (single/low copy vs. high copy) a different estimate of the relationships may be obtained (Powell *et al* 1996).

On the other hand, Table (7) revealed that the similarity percentage (86.2% and 87.5%) was high between the control and the concentration of colchicine of 0.004 ppm with Zebda and the 0.002 ppm concentration with Company respectively.

Table 7. Genetic similarity matrices computed according to Dice coefficients from Zebda and Company varieties treated with different concentration of sodium azide and cholchicine

Zecon												
ZeS.002	85.6											
ZeS.004	82.3	80.1										
ZeS.008	79.3	80.2	83.5									
ZeC.002	80.9	77.1	76.1	70.2								
ZeC.004	89.2	75.2	70.3	74.5	86.2							
ZeC.008	88.5	73.1	72.1	68.3	82.3	85.2						
Copcon	80.6	75.2	79.8	65.1	70.2	69.5	60.8					
CopS.002	78.1	64.1	65.1	59.0	62.1	68.1	73.1	81.3				
CopS.004	75.1	66.2	58.1	65.1	69.1	64.9	66.4	80.6	77.1			
CopS.008	69.2	65.1	67.3	62.3	64.1	62.3	66.7	70.2	66.1	79.1		
CopC.002	66.1	60.9	62.1	58.1	55.2	61.3	57.1	53.1	69.3	80.9	87.5	
CopC.004	58.8	64.3	53.1	42.5	50.2	62.1	68.5	75.2	60.3	59.1	55.4	85.9
CopC.008	40.9	46.3	55.8	60.9	55.3	68.3	75.9	70.1	75.2	71.3	89.5	83.5	75.5

In conclusion, the effect of the chemical mutagens differ from one mango genotype to another, i.e is dependent on the genetic background of the genotype, the concentration of chemical mutagen and its mode of action in the cell.

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تأثير الصوديوم آزايد والكولشيسين على صنفى المانجو الزبدة والكبانية

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أجرى هذا البحث فى صوبة معهد بحوث البساتين خلال موسمي الزراعة 2005/2004 و 2006/2005 لدراسة تأثير استخدام بعض المطفرات الكيماوية (صوديوم آزايد و كولشيسين) على الأختلافات الوراثية للشتلات النيوسيلية لصنفي المانجو الزبدة و الكوبانيه.

تم استخدام طريقتين للاضافة اما اضافة ارضية بتركيزات من صفر , 0.2, 0.4 0.8% مع الرش بتركيزات من صفر, 0.002, 0.004, 0.008 جزء في المليون لكلاً المادتين الكيماويتين او استخدام الرش فقط بتركيزات من صفر, 0.002, 0.004, 0.008 جزء في المليون وأظهرت النتائج:

- أن الإضافة الأرضية بتركيز 0.8%مع الرش بتركيز 0.008 جزء في المليون للصوديوم آزايد أدى لتقصير طول النبات معنوياً بينما أدى لزيادة سمك الساق في كلا الصنفين الزبدة و الكوبانيه.
- أدت جميع المعاملات بالصوديوم أزايد والكولشيسين لتقليل عدد الأوراق معنوياً لكل من الزبدة و الكوبانيه بينما أعطى الرش فقط بالصوديوم آزايد بتركيز 0.008 جزء في المليون الى زيادة مساحة الورقة للزبدة في حين أن تركيز 0.004 جزء في المليون من نفس المطفر كان أفضل للكوبانية.

- وجد أن رش الصوديوم آزايد بتركيز 0.002 جزء في المليون على الشتلات النيوسيلية للمانجو صنف الزبدة أدى لزيادة محتوى الأوراق من كلوروفيل A و الكاروتينات بينما زاد محتوى الأوراق من كلوروفيل B عند الرش بالكولشيسين بتركيز 0.004 جزء في المليون.
- زاد محتوى أوراق الشتلات النيوسيلية للمانجو صنف الكوبانيه من كلوروفيل A,B و الكاروتينات عند استخدام الصوديوم آزيد بتركيز 0.2% اضافة أرضية مع الرش بتركيز 0.002 جزء في المليون.
- الإضافة الأرضية للصوديوم آزايد بتركيز 0.8% مع الرش بتركيز 0.008 جزء في المليون أدى لزيادة محتوى أوراق شتلات الزبدة من الاندولات بينما أدى استخدام الصوديوم آزايد بتركيز 0.008 جزء في المليون رشاً فقط الى زيادة محتوى الأوراق من الفينولات.
- أدت جميع المعاملات الى نقص محتوى أوراق الكوبانيه من الاندولات بينما زادت الفينولات عند استخدام الاضافة الأرضية للصوديوم آزايد بتركيز 0.8% مع الرش بتركيز 0.008 جزء في المليون.
- على المستوى الجزيئي، لم يكن هناك اختلاف معنوي بين الزبدة و الكوبانيه في ظهور أو اختفاء حزم جديدة من الـDNA عند استخدام مادة الصوديوم أزايد مع التركيزات المختلفة. من ناحية أخرى، كان صنف الكوبانية أكثر تأثراً بالكولشيسين عند استخدامه بالتركيزات المختلفة و ذلك سواء كان ذلك بظهور حزم جديدة أو اختفاء حزم الـ DNA القديمة.بالاضافة إلى أن درجة التشابه بعد استخدام المادتين المطفرتين (الكولشيسن و الصوديوم أزايد) أوضحت اختلافات كبيرة في درجة التشابه الوراثى عند استخدام مع التركيزات المختلفة أو الأصناف المختبرة المختلفة.