RESULTS AND DISCUSSION

4-RESULTS AND DISCUSSION

1 - *Ceratonia siliqua*

1-a-Effect of germplasm preservation treatments on physical characters

Data in **Table (3)** show the effect of some germplasm preservation treatments and storage duration on some physical characters of *Ceratonia siliqua* explants. It is clear from **Table (3-A)** that using coumarin at 1.5 mg/l induced highly significant increase in survival percentage followed by PEG at 15 g/l as well as PEG at 20 g/l then coumarin at (0.5 and 1.0 mg/l) and L.P.W. overlaying as well as PEG at 10 g/l as compared to the other used treatments and the control. Meanwhile, significant increase of callusing percentage and moisture contents of shoots percentage and moisture contents of callus percentage occurred when L.P.W. overlaying was used as compared with the other used treatments and the control. However, using desiccation had significantly increased number of shoots as well as L.P.W. overlaying followed by L.P.W soaking as compared with the other used treatments and the control. On the other hand, significant increase of shoots length values occurred when coumarin at different concentrations were used as compared with the other used treatments and the control .Meanwhile, the highest number of leaves (8.47) was recorded by L.P.W. soaking. However, using low temperature and encapsulation as preservation treatments induced the lowest significant increase in callusing, shoot number, shoot length, leaves number and callus moisture contents percentage.

Table (3–B) indicates that increasing duration from 6 to 18 months reduced most parameters under investigation

(survival percentage, callusing percentage and shoot M.C. percentage). However, preservation for 12 months maximized shoot number, shoot length and number of leaves as compared with either 6 or 18 months.

Table (3): Effect of germplasm preservation treatments and storage durations on some physical characters of *in vitro* *Ceratonia siliqua* explants.

Table (3-A): Effect of germplasm conservation treatments.

Trealments	Survival %	Callusing %	Shoot No.	Shoot length cm	Leaves No.	Shoots M.C.	Callus M.C.
Control	20.66	30.71	1.19	0.91	4.96	68.70	69.71
Low temperature 4°C	61.33	00	1.11	0.41	2.01	59.90	00
P.E.G. 10 g/l	85.33	53.08	1.64	0.78	5.72	71.52	53.08
P.E.G. 15 g/l	92	57.71	1.69	0.77	4.90	61.92	57.71
P.E.G. 20 g/l	90.66	55.574	1.56	0.62	4.95	72.64	55.74
Desiccation	77.77	73.89	2.68	0.98	4.73	57.53	73.89
L.P.W. Soaking	82.66	78.58	2.24	0.97	8.47	77.19	78.58
L.P.W Overlaying	86.66	86.73	2.61	0.41	5.90	87.54	86.73
Coumarin 0.5 mg/l	88	75.09	1.67	1.34	8.06	72.13	75.01
Coumarin 1.0 mg/l	88	76.35	1.84	1.26	6.82	72.67	76.35
Coumarin 1.5 mg/l	93.33	81.78	1.64	1.68	6.99	69.54	81.78
Sorbitol 5g /l	53.33	46.40	1.05	0.71	3.85	48.78	46.71
Sorbitol 10g /l	52.00	38.802	1.02	0.47	4.30	48.64	38.80
Sorbitol 15g /l	60.00	48.806	1.21	0.49	4.38	50.12	48.81
Encapsulation	26.666	00	0	0	0	27.62	00
L . S . D. 5 %	**3.73**	**4.33**	**0.13**	**0.07**	**0.28**	**1.94**	**4.33**

Table (3-b): Effect of storage duration

Treatments	Survival %	Callusing %	Shoot No.	Shoot length cm	Leaves No.	Shoot M.C.	Callus M.C.
6 months	92.88	79.66	1.63	0.81	5.19	75.30	59.37
12 months	82.13	78.88	1.85	0.98	6.02	68.07	65.34
18 months	49.86	65.13	1.34	0.58	4.01	45.92	43.81
L.S.D at 5%	**0.75**	**1.02**	**0.04**	**0.03**	**0.09**	**0.65**	**0.87**

Furthermore, **Table (3–C)** reflects that P.E.G. at 15 g/l, L.P.W. soaking and L.P.W. overlaying treatments after 6 months as well as L.P.W. soaking, and coumarin at different concentrations after 12 months induced significant increase in survival percentage as compared with the other combinations in this respect.

Meanwhile, the interaction between preservation treatments and storage duration were more or less similar from statistical point of view when callusing percentage was concerned. On contrast, using desiccation treatment after 18 months induced significantly increase in shoot number followed by L.P.W. soaking after 18 months and coumarin at 5 mg /l after 12 months as well as L.P.W. overlaying after 6 months then P.E.G. at 10 g/l after 6 months and sorbitol at 15 g/l after 12 months as compared to the other combinations in this respect. Meanwhile, significant increase of shoot length values occurred when coumarin at 0.5 mg/l was used after 12 months as compared the other combinations used and the control. However, the highest number of leaves was formed after 12 months when soaking the explains on liquid paraffin but, the lowest value recorded when stored under low temperature (4°C) after 6 months. On the other hand, the interaction between germplasm preservation treatments and storage duration was more or less similar from statistical point of view when moisture content of shoots and callus were considered.

Table (4): Effect of germplasm conservation treatments and storage durations on some chemical characters of *Ceratonia siliqua* explants.

Table (4-A): Effect of germplasm conservation treatments.

Treatments	Chlorophyll A Mg/100g f.w.	Chlorophyll B Mg/100g f.w	Carotenoids Mg/100g f.w	Total Phenols Mg/100g f.w	Total Indols Mg/100g f.w	Total Carbohydrate Mg/100g f.w	T.S.S %	Osmotic Potentiality Atm.	PPO U/ml.min	POD U/ml.mi
Control	39..97	16.01	51.70	21.81	172.15	18.71	13.51	11.81	4.91	9.48
Low temperature 4°C	35.59	9.85	28.64	5.55	65.48	11.38	3.47	2.74	2.61	27.82
P.E.G. 10 g/l	38.59	27.58	56.84	17.97	164.39	24.55	18.09	16.84	4.98	18.31
P.E.G. 15 g/l	55..9	21.49	77.62	9.59	117.66	25.71	17.38	18.17	18.74	56.26
P.E.G. 20 g/l	35.89	19.25	55.38	12.30	181.37	25.39	17.62	16.28	7.04	43.26
Desiccation	69.88	32.81	94.46	40.91	150.52	27.43	18.53	17.83	33.61	172.57
L.P.W. Soaking	45.52	18.88	50.34	2.96	66.25	8.97	12.09	10.13	3.33	104.92
L.P.W Overlaying	88.09	56.27	71.26	1.33	93.15	25.74	7.16	7.21	2.46	29.02
Coumarin 0.5mg/l	77.24	44.6	118.76	11.83	144.75	27.63	13.00	11.21	4.16	28.29
Coumarin 1.0mg/l	68.31	41.79	88.20	17.65	131.83	14.10	10.33	8.60	5.63	12.09
Coumarin 1.5mg/l	85.05	55.95	149.89	9.46	156.46	18.76	11.24	9.41	10.21	14.43
Sorbitol 5g /l	99.27	39.02	113.40	9.17	79.91	13.10	6.69	5.52	2.07	9.38
Sorbitol 10g /l	49.94	20.77	63.99	4.13	125.83	15.41	9.78	8.57	1.87	8.37
Sorbitol 15g /l	117.23	62.96	102.10	6.10	89.35	22.12	9.58	8.363	1.65	11.24
Encapsulation	16.14	12.79	19.36	1.78	47.40	9.44	8.09	8.25	2.07	23.97
L.S.D. at 5 %	9.72	50.01	9.42	1.82	10.30	2.62	1.91	3.07	----------	--------

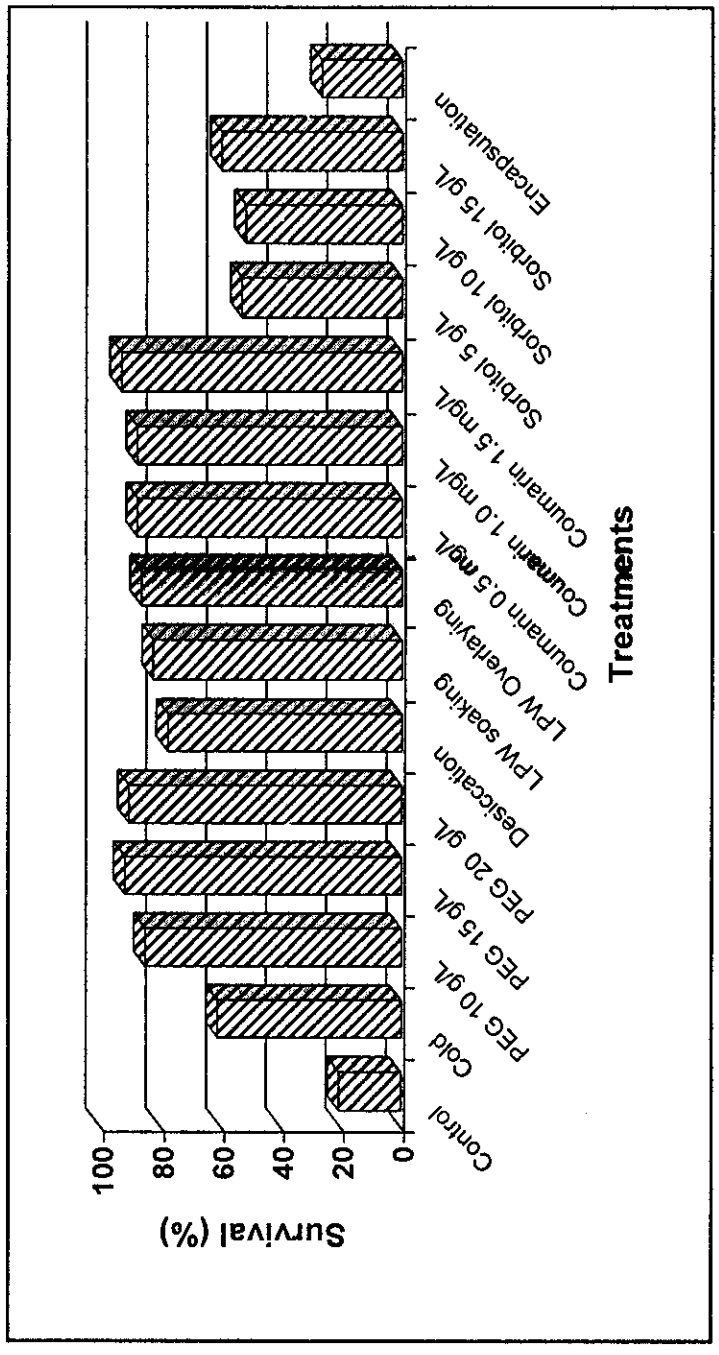

Fig. (1): Effect of germplasm preservation treatments on survival rate of *Ceratonia siliqua* explants *in vitro* after storage duration.

1-b- Effect of germplasm preservation treatments on chemical characters

Table (4-A) shows the effect of some germplasm preservations treatments on the chemical characters of *Ceratonia siliqua* explants. It is quite evident that using sorbitol at 15 g/l gave significantly the highest chlorophyll A and B as compared with the other treatments and the control. Besides, using coumarin at 1.5 mg/l induced the highest carotenoids followed by coumarin at 0.5 mg/l and sorbitol at 5 g/l among the other treatments used. Meanwhile, desiccation treatment succeeded in increasing total phenols values as compared with the other used treatments and the control. However, significant increase of total indols occurred when P.E.G. at 15 g/l was used. Furthermore, using coumarin at 0.5 mg/l as well as desiccation treatment caused the highest effect in increasing total sugars followed by using P.E.G. at 10 , 15 and 20 g/l and L.P.W. overlaying as well as sorbitol at 15 g/l as compared with the other used treatments and the control in a descending order. Moreover, significant increase occurred in both T.S.S and osmotic potentiality contents when P.E.G. at (10, 15 and 20 g/l) as well as desiccation treatment were used. On the other hand, significant differences were lacked among different treatments under study when polyphenol oxidase and peroxidase parameters were concemed. Comparing different storage duration **Table (4-B)** indicates that all chemical characters were decreased by increasing storage duration from 6 to 12 and 18 months except total indoles and T.S.S. parameters

Table (4): Effect of germplasm conservation treatments and storage durations on some chemical characters of *Ceratonia siliqua* explants.

Table (4-A): Effect of germplasm conservation treatments.

Treatments	Chlorophyll A Mg/100g f.w.	Chlorophyll B Mg/100g f.w	Carotenoids Mg/100g f.w	Total Phenols Mg/100g f.w	Total Indols Mg/100g f.w	Total Carbohydrate Mg/100g f.w	T.S.S %	Osmotic Potentiality Atm.	PPO U/ml.min	POD U/ml.mi
Control	39..97	16.01	51.70	21.81	172.15	18.71	13.51	11.81	4.91	9.48
Low temperature 4°C	35.59	9.85	28.64	5.55	65.48	11.38	3.47	2.74	2.61	27.82
P.E.G. 10 g/l	38.59	27.58	56.84	17.97	164.39	24.55	18.09	16.84	4.98	18.31
P.E.G. 15 g/l	55..9	21.49	77.62	9.59	117.66	25.71	17.38	18.17	18.74	56.26
P.E.G. 20 g/l	35.89	19.25	55.38	12.30	181.37	25.39	17.62	16.28	7.04	43.26
Desiccation	69.88	32.81	94.46	40.91	150.52	27.43	18.53	17.83	33.61	172.57
L.P.W. Soaking	45.52	18.88	50.34	2.96	66.25	8.97	12.09	10.13	3.33	104.92
L.P.W Overlaying	88.09	56.27	71.26	1.33	93.15	25.74	7.16	7.21	2.46	29.02
Coumarin 0.5mg/l	77.24	44.6	118.76	11.83	144.75	27.63	13.00	11.21	4.16	28.29
Coumarin 1.0mg/l	68.31	41.79	88.20	17.65	131.83	14.10	10.33	8.60	5.63	12.09
Coumarin 1.5mg/l	85.05	55.95	149.89	9.46	156.46	18.76	11.24	9.41	10.21	14.43
Sorbitol 5g /l	99.27	39.02	113.40	9.17	79.91	13.10	6.69	5.52	2.07	9.38
Sorbitol 10g /l	49.94	20.77	63.99	4.13	125.83	15.41	9.78	8.57	1.87	8.37
Sorbitol 15g /l	117.23	62.96	102.10	6.10	89.35	22.12	9.58	8.363	1.65	11.24
Encapsulation	16.14	12.79	19.36	1.78	47.40	9.44	8.09	8.25	2.07	23.97
L.S.D. at 5 %	9.72	50.01	9.42	1.82	10.30	2.62	1.91	3.07	---------	--------

Table (4-B): Effect of storage duration of germplasm conservation.

Treatments	Chlorophyll A Mg/100g f.w	Chlorophyll B Mg/100g f.w	Carotenoids Mg/100g f.w	Total Phenols Mg/100g f.w	Total Indols Mg/100g f.w	Total Carbohydrates Mg/100g f.w	T.S.S %	Osmotic Potentiality Atm.	PPO U/ml.min	POD U/ml.min
6 months	99.24	47.82	113.90	12.72	113.71	26.92	12.91	12.15	7.58	49.13
12 months	53.30	35.76	70.98	8.97	137.48	15.19	13.60	12.08	7.07	39.56
18 months	34.38	14.82	45.91	8.12	108.51	17.98	11.20	10.36	6.41	24.72
L.S.D at 5 %	4.34	22.36	4.21	0.81	4.60	1.17	0.85	1.37	-----	----

T.S.S. and total indols were significantly increased after 12 months followed by 6 months as compared with 18 months in a descending order. On the other hand, no significant difference was observed between different storage duration when polyphenol oxidase and peroxidase parameters were considered. Regarding different interactions **Table (4-C)** verifies that using sorbitol at 5 and 15 g/l as well as L.P.W. overlaying after 6 months were statistically increased chlorophyll A. However, a significant increase in chlorophyll B values was reduced when L.P.W. overlay after 6 months was used. Furthermore, using sorbitol treatment at 5 g/l increased carotenoids followed by using sorbitol at 15 g/l after 6 months then coumarin at 1.5 mg/l after 12 months as compared to the other combinations used and the control. Meanwhile, significant increase of total phenols contents occurred when coumarin at 0.5 mg/l preserved for 6 months was used. However, using sorbitol at 10 g/l after 12 months as well as P.E.G at 20g/l after 18 months had higher total indols values as compared with the other combinations in this respect. Furthermore, using L.P.W. overlay after 18 months increased total carbohydrates statistically followed by using coumarin at 0.5 mg/l after 18 months as well as using sorbitol at 15 and P.E.G at 20g/l after 6 months then desiccation as compared with the other combinations used and the control. On contrast, using desiccation treatment after 18 months significantly surpassed other combinations in improving T.S.S. However, significant increase in osmotic compared to with without cold preservation. Inanition; **Yoshihara *et al.* (1996)** showed that on lilium bulb scale under control conditions the sugars and starch were decreased during days 10–42 of storage at 5 or 25°C for 180 days.

Table (4-C1): Effect of interaction between preservation treatments and storage durations.

Treatments	Chlorophyll - A			Chlorophyll - B			Carotenoids			Total Phenols			Total Indols		
	6	12	18	6	12	18	6	12	18	6	12	18	6	12	18
Control	36.53	51.42	31.96	17.10	15.75	15.18	47.71	52.77	54.60	25.16	21.29	19.00	93.29	197.56	225.61
Cold	40.64	66.14	0.00	14.40	15.14	0.00	38.53	47.38	0.00	11.84	4.80	0.00	105.60	90.84	0.00
P.E.G 10 g/l	46.68	28.00	41.07	46.79	21.94	14.01	67.41	43.88	59.24	20.44	19.38	14.09	134.99	205.97	152.19
P.E.G 15 g/l	57.04	71.08	39.57	23.39	28.01	13.07	95.90	74.43	62.51	12.76	7.55	8.45	101.45	101.21	150.32
P.E.G 20 g/l	65.21	14.73	27.72	32.97	15.62	9.16	79.39	29.10	57.65	16.30	9.21	11.39	125.20	170.24	248.66
Desiccation	142.29	25.24	42.11	64.57	19.30	14.58	176.38	52.99	54.02	9.71	2.12	2.92	211.13	112.51	127.92
L.P.W. Soaking	35.17	69.06	32.35	15.22	22.00	19.40	50.31	49.11	51.59	2.30	4.63	1.95	45.73	99.87	53.16
L.P.W. overlay	198.64	45.10	20.52	135.43	25.19	8.19	139.13	54.72	19.94	1.26	1.99	0.76	79.58	120.60	79.26
Coumarin 0.5mg/l	71.46	68.88	91.36	49.67	39.31	44.82	135.35	105.41	115.52	6.51	11.65	17.32	82.18	145.85	206.22
Coumarin 1.0mg/l	53.47	109.11	42.36	23.68	86.53	15.16	82.09	120.53	61.99	23.63	17.40	11.93	59.26	155.48	180.73
Coumarin 1.5mg/l	38.47	88.00	128.68	22.13	95.02	50.70	87.54	228.60	133.54	2.52	9.90	15.97	116.58	167.19	185.61
Sorbitol 5g/l	274.65	23.17	0.00	106.98	10.07	0.00	290.25	49.95	0.00	21.39	6.12	0.00	133.41	106.33	0.00
Sorbitol 10 g/l	99.22	50.61	0.00	33.01	29.30	0.00	120.16	71.80	0.00	9.47	2.92	0.00	124.99	252.50	0.00
Sorbitol 15g/l	274.65	77.05	0.00	87.61	101.27	0.00	234.24	72.05	0.00	16.18	3.62	0.00	144.02	124.02	0.00
Encapsulation	48.43	0.00	0.00	38.38	0.00	0.00	58.09	0.00	0.00	5.33	0.00	0.00	142.19	0.00	0.00
L.S.D 5 %	16.84			86.63			16.32			3.15			17.85		

Meanwhile, the highest percentage of survival percentage was observed in coumarin at different concentrations as compared with the other combinations. However, significant increase of shoot number occurred when coumarin at 0.5mg/l was used after 12 months followed by L.P.W. soaking as well as coumarin at 0.5 mg/l after 18 months then L.P.W. soaking after 12 months as compared with the other combinations used and the control .On the contrast, using L.P.W. soaking after 6 months induced significant increase in shoot length followed by soaking and coumarin at 0.5 and 1.5 mg/l treatments after 18 months as compared with other combinations. Furthermore, significant increase of number of leaves occurred when sorbitol (at 10 and 15 g/l) and coumarin (at 0.5 mg/l) as well as desiccation and L.P.W. soaking after 12 months and coumarin (at 0.5 mg/l) after18 months as well as P.E.G. at 15 g/l after 6 months as compared with the other combinations used and the control. These results are in agreement with the findings of **Sumaryat *et al.* (1992)** they found that when used PEG (6000) 2% in culture media of colonies of haploid *N. plumbaginfolia* leaves which produced. One resistance line from each treatments was regenerated into plants. Also, **Eliasson *et.al.*(1994)** they stated that culturing of *Prunus seratonia* var. *virens* shoot explants on medium supplemented with paclobutrazol maximized survivals after 4 weeks.

Table (5): Effect of germplasm preservation treatments and storage durations on viability and some growth characters of regenerated *in vitro Ceratonia siliqua* explants *in vitro*.

Table (5-A): Effect of germplasm preservation treatments.

treatments	Viability %	Shoot No.	Shoot length cm	Leaves No.
Control	80.00	1.31	0.59	4.03
Low temperature 4°C	38.67	0.84	0.35	3.16
P.E.G. 10 g/l	77.33	1.52	0.51	4.12
P.E.G. 15 g/l	81.33	1.24	0.54	4.16
P.E.G. 20 g/l	85.33	1.30	0.60	4.20
Desiccation	73.33	1.70	0.47	4.44
L.P.W. Soaking	90.67	2.08	0.88	4.89
L.P.W Overlaying	84.00	1.94	0.41	3.91
Coumarin 0.5 mg/l	96.00	1.36	0.67	4.78
Coumarin 1.0 mg/l	100.00	2.05	0.63	4.77
Coumarin 1.5 mg/l	93.33	1.33	0.67	4.32
Sorbitol 5g /l	64.00	1.22	0.30	2.78
Sorbitol 10g /l	58.67	1.13	0.40	3.15
Sorbitol 15g /l	61.33	1.00	0.43	2.97
Encapsulation	18.67	0.43	0.14	0.22
L . S . D. 5 %	**3.79**	**0.11**	**0.04**	**0.13**

Table (5-B): Effect of storage duration.

Treatments	Survival %	Shoot No.	Shoot length cm	Leaves No.
6 months	85.60	1.43	0.56	4.14
12 months	76.53	1.48	0.56	4.29
18 months	58.40	1.18	0.40	2.76
L.S.D at 5 %	**0.760**	**0.022**	**0.009**	**0.027**

2–*Acacia slicina* L.

2-a- Effect of germplasm preservation treatments on physical characters.

Data in **Table (6)** show the effect of germplasm preservation treatments and storage durations on physical characters of *Acacia salicina* L. explants .It is clear from **Table (6-A)** that supplementation of coumarin at 0.5 , 1.0 and 1.5 mg/l as well as PEG at 10 and 20 g/l and sorbitol 5 g/l had a positive effect increasing survival percentage followed by L.P.W soaking and sorbitol at 10 g/l then PEG 15 g/l as compared to the other germplasm preservation treatments and control in a descending order. Meanwhile, significant increase of callus percentage occurred when coumarin at 0.5, 1.0 and 1.5 mg/l, sorbitol at 5 and 15 g/l and desiccation as well as L.P.W. overlaying were used in comparison with the other used treatments and the control. However, coumarin at 1.0 mg/l and PEG at 10 g/l as well as 5 g/l sorbitol succeeded in increasing shoot number followed by coumarin at 0.5 mg/l and 15 g/l PEG then L.P.W. soaking as well as 15 g/l sorbitol as compared to the other used treatments and the control. Furthermore, supplementation of 5 g/l sorbitol induced significant increase in average shoot length followed by coumarin at 1.5, 0.5 and 1.0 mg/l respectively as compared with the other germplasm preservation treatments and the control. However, addition of either coumarin 0.5, 1.0 or 1.5 mg/l to the culture medium significantly increased leaves number followed by L.P.W/ soaking as compared with the other germplasm treatments and control . Moreover, significant decrease of M.C. percentage of shoots and callus occurred when encapsulation treatment and low temperature (4°C) in the dark were used as compared with the other used treatments and the control.

Table (6): Effect of germplasm preservation treatments and storage durations on some physical characters of *in vitro Acacia salicina*.

Table (6-A): Effect of germplasm preservation treatments.

Treatments	Survival %	Callusing %	Shoot No.	Shoot length cm	Leaves No.	Shoots M.C.	Callus M.C.
Control	17.33	72	3.83	0.89	5.84	80.90	67.97
Low temperature 4°C	52	4	0.44	0.27	1.86	56.22	00.00
P.E.G. 10 g/l	97.33	92.67	5.02	0.83	4.02	77.52	81.46
P.E.G. 15 g/l	88	90.67	4.27	0.89	4.05	7740	66.77
P.E.G. 20 g/l	96	93.33	3.02	0.65	4.42	77.43	54.43
Desiccation	74.46	100	2.72	1.05	5.06	59.39	70.21
L.P.W. Soaking	93.33	98.67	4.04	0.77	6.15	79.54	79.44
L.P.W Overlaying	79.99	100	1.87	0.64	5.49	87.34	87.75
Coumarin 0.5 mg/l	100	100	4.85	1.29	6.29	77.88	83.37
Coumarin 1.0 mg/l	98.66	100	5.24	1.26	6.32	78.13	81.99
Coumarin 1.5 mg/l	97.33	100	4.41	1.64	7.72	75.60	85.03
Sorbitol 5g /l	96	100	5.02	2.94	5.63	78.65	87.12
Sorbitol 10g /l	93.33	94.67	3.99	0.93	5.61	77.83	77.36
Sorbitol 15g /l	82.66	100	4.02	0.79	4.41	80.73	82.23
Encapsulation	25.33	2.67	00	00	00	29.47	00.00
L.S.D. at 5%	3.47	2.86	0.23	0.06	0.24	1.61	3.61

Table (6–B) indicates that the survival and callus percentages as well as number of leaves and M.C. percentage of shoots had indirect relationship with storage duration as they decreased with increasing the storage period from 6 to 12 and 18 months in a descending order. On the other hand, significant increase of shoot number, shoot length and M.C. of callus

occurred when duration storage was increased from 6 to 12 months, then decreased again by increasing storage duration up to 18 months.

Table (6-b): Effect of storage duration

Treatments	Longevity %	Callusing %	Shoot No.	Shoot length cm	Leaves No.	Shoot M.C.	Callus M.C.
6 months	92.88	79.66	1.63	0.81	5.19	75.30	59.37
12 months	82.13	78.88	1.85	0.98	6.02	68.07	65.34
18 months	49.86	65.13	1.34	0.58	4.01	45.92	43.81
L.S.D at 5%	0.75	1.02	0.04	0.02	0.09	0.65	0.87

Dealing with the interactions between germplasm preservation treatments and storage durations it appears from **Table (6–C)** that the survival and callusing percentages were more or less similar from statistical point of view in all storage duration and germplasm preservation treatments. Meanwhile, the highest values of shoot number were recorded with coumarin at 1.0 mg/l after 12 months as well as sorbitol 5 g/l after 6 months then followed by 10 g/l PEG and coumarin at 0.5 mg/l after 6 months as well as coumarin at 1.5 mg/l after 12 months then sorbitol at 5 g/l after 12 months as compared with the other used treatments and the control. However, significant increase of shoot length occurred when coumarin at 1.5 mg/l after 6 months as well as sorbitol at 5 g/l after 12 months were used .On the other hand, the highest number of leaves was recorded with coumarin at 1.5 mg/l after 6 months followed by coumarin at 1.0 mg/l after 12 and 18 months as compared with the other combinations under study. However, significant increase of shoots and M.C. percentage of callus occurred when L.P.W. overlay was used after 6 months.

Table (6-C): Effect of interaction between conservation treatments and storage durations.

Treatments	Survival %			Callusing %			Shoot Number			Shoot Length cm			Leaves Number		
	6	12	18	6	12	18	6	12	18	6	12	18	6	12	18
Control	20	20	10	96	100	100	2	2.43	2.15	1.06	1.09	0.58	4.62	5.81	4.47
Cold	92	92	0	29	8	0	1.66	1.68	0	0.50	0.72	00	2.87	3.14	00
P.E.G 10 g/l	92	92	72	86	92	96	2.17	1.77	1.0	0.43	0.95	0.97	5.08	5.61	6.47
P.E.G 15 g/l	100	84	92	96	100	100	1.5	1.92	1.66	0.64	0.96	0.71	4.36	4.89	5.44
P.E.G 20 g/l	92	88	92	83	100	85	1.65	1.7	1.33	0.49	0.71	0.66	4.12	4.97	5.77
Desiccation	93.33	73.33	66.66	71.99	100	100	1.62	2.2	4.23	1.39	1.14	0.42	3.85	7.06	3.28
L.P.W. Soaking	100	100	48	100	100	100	2.04	1.92	2.76	1.11	1.08	0.73	6.73	11.54	7.14
L.P.W. overlay	100	86.66	73.33	100	53.33	100	2.53	3.1	2.2	00	0.66	0.57	5.76	6.19	5.75
Coumarin 0.5mg/l	88	100	76	95	100	96	1.62	1.8	1.61	1.44	1.28	1.31	7.96	8.36	7.86
Coumarin 1.0mg/l	96	92	76	96	100	100	1.49	2.73	1.31	1.07	1.43	1.28	5.96	6.94	7.56
Coumarin 1.5mg/l	92	100	88	100	100	100	1.61	1.36	1.96	1.03	2.46	1.54	6.65	8.01	6.31
Sorbitol 5g/l	88	72	0	100	85	0	1.62	1.55	0	1.23	0.92	00	5.79	5.77	00
Sorbitol 10 g/l	88	68	0	55	63	0	1.6	1.47	0	0.66	0.77	00	7.18	5.72	00
Sorbitol 15g/l	92	88	0	87	82	0	1.43	2.21	0	0.93	0.54	00	6.94	6.21	00
Encapsulation	80	0	0	00	0	0	0	0	0	00	00	00	00	00	00
L.S.D at 5 %	11.21			9.17			0.40			0.20			0.84		

The above mentioned results in general agreement with the findings of **Samsonova and Trushechkin (1990)** They reported that storing of 20 *Faragaria ananassa* cv. Meristems at 2 - 6°C resulted in varying the viability of the plants with cultivars and decreased with increasing storage duration 4 – 12 months. In addition, **Janeiro *et al.* (1995)** exposed *Camellia reticulata* and *C.japonica* somatic embryos to 3 – 4°C for 3 – 12 months. They found that the number of surviving embryos/cluster decreased by increasing cold storage durations from 3 to 12 months. Also, assured the findings of **Wang (1999)** who stated that culturing of *in vitro Lilium longflorium* cultivars New Bao on medium supplemented with paclobutrazol gave a good leaf formation compared to the control.

Table (6-C2): Effect of interaction between preservation treatments and storage durations.

Treatments	Shoot M.C.			Callus M.C.		
	6	12	18	6	12	18
Control	73.92	69.61	62.57	88.80	79.57	40.76
Cold	89.87	89.82	00	00	00	00
P.E.G 10 g/l	76.34	68.30	69.92	32.4	58.63	68.22
P.E.G 15 g/l	67.51	58.13	60.1	44.79	71.94	56.40
P.E.G 20 g/l	72.23	60.60	85.11	64.58	71.45	31.19
Desiccation	60.54	65.94	46.11	79.06	77.67	64.94
L.P.W. Soaking	85.12	81.36	65.09	84.41	80.77	70.56
L.P.W. overlay	87.49	87.16	87.98	80.6	88.11	91.48
Coumarin 0.5mg/l	74.86	68.39	73.13	76.82	72.48	75.71
Coumarin 0.5mg/l	72.40	75.14	70.45	74.17	81.91	72.98
Coumarin 0.5mg/l	67.34	72.95	68.32	76.26	84.13	48.94
Sorbitol 5g/l	73.31	73.04	00	70.04	69.18	00
Sorbitol 10 g/l	72.61	73.31	00	44.44	71.96	00
Sorbitol 15g/l	73.11	77.24	00	74.16	72.25	00
Encapsulation	82.86	00	00	00	00	00
L.S.D at 5%		5.81			13.00	

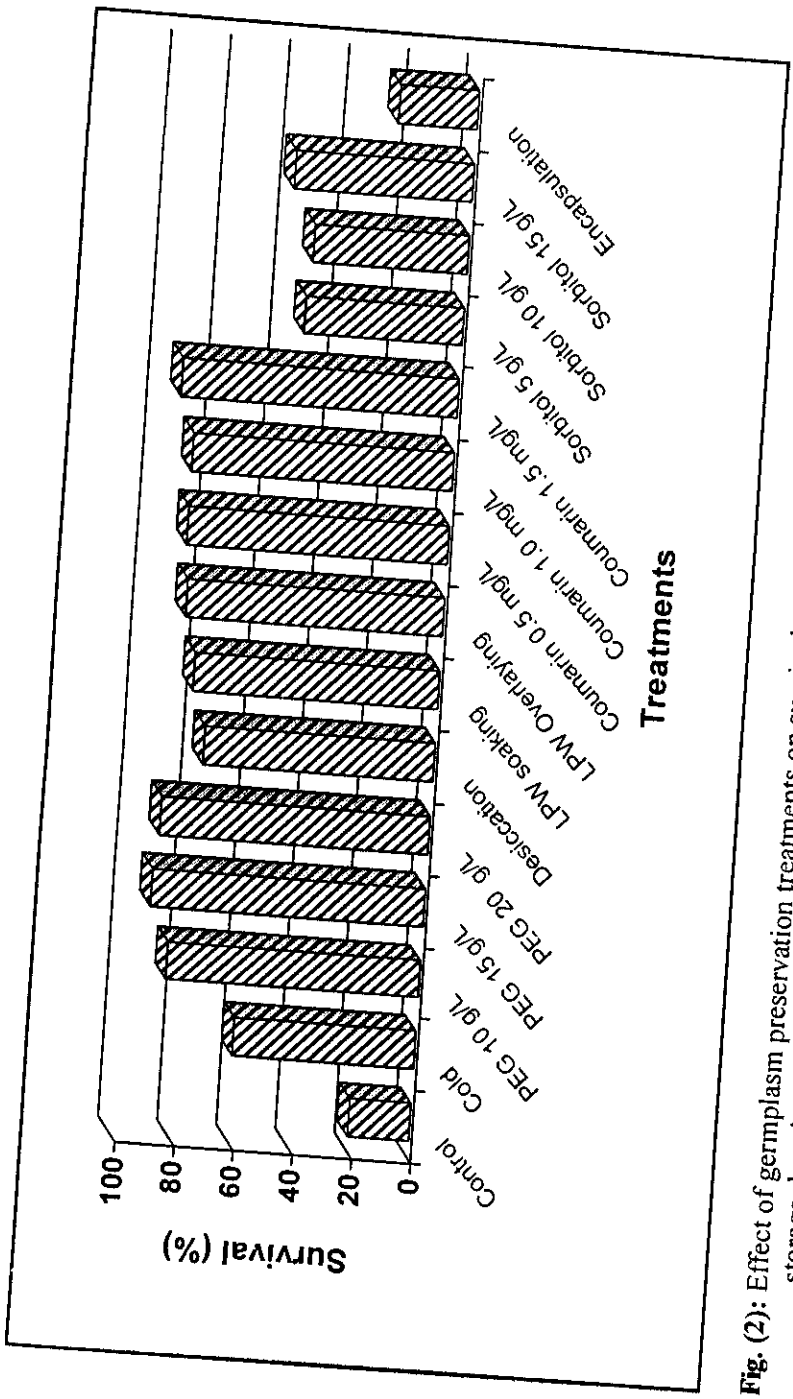

Fig. (2): Effect of germplasm preservation treatments on survival rate of *Acacia salicina* explants *in vitro* after storage duration.

2-b. Effect of germplasm preservation treatments on chemical characters.

Table (7) showed the effect of germplasm preservation treatments and storage durations on chemical characters of *in vitro Acacia salicina* explants .It is clear from **Table (7-A)** that L.P.W. overlaying induced significant increase in chlorophyll A and B as well as carotenoids as compared with the other used treatments and control. Meanwhile, significant increase of total phenols occurred when 15 g/l PEG was used followed by 0.5 and 1.5 mg/l coumarin, control, 10 g/l PEG and 15 g/l sorbitol as compared to the other used treatments. However, coumarin at 1.5 mg/l induced significant increase in total indols followed by PEG at 15 mg/l then coumarin at 0.5 mg/l as compared to the other used treatments and control. Furthermore, total carbohydrates and peroxidase were significantly increased when desiccation was used. However, lower sorbitol at 5 g/l significantly increased total soluble solids percentage in relation to the other studied treatments and control. On the other hand, sorbitol at 10 and 15 g/l as well as PEG at 10 g/l and desiccation gave the highest significant osmotic potentiality followed by PEG at 15 and 20 g/l L.P.W. soaking, 0.5 mg/l coumarin, 5g/l sorbitol and control as compared with the other used treatments. However, using of 10 g/l PEG succeeded increasing polyphenol oxidase followed by L.P.W. overlaying then PEG at 20 g/l and desiccation as compared with the other used treatments and control.

Concerning **Table (7–B)** it is clear that chemical characters *i.e.* chlorophyll A & B, carotenoids, total carbohydrates, T.S.S. and peroxidase were significantly decreased with increasing storage duration. However, the reverse was true with indols as well as polyphenol oxidase parameters.

Dealing with the interaction between germplasm treatments and storage durations as shown in **Table (7–C)** *it is* obvious that both liquid paraffin wax overlaying at room temperature after 6 months as well PEG at 15 g/l after 12 months increased chlorophyll A content and carotenoids as compared with the other combinations. Meanwhile, significant increase of chlorophyll B content occurred when liquid paraffin wax overlaying after 6 months was used as compared with the other combinations. On contrast, PEG at 10 g/l stored for 6 months as well as sorbitol at 5 g/l stored for 18 months induced significant increase in total phenols values as compared to the other combinations used under study. On the other hand, storing of the explants under room temperature (control) for 18 months induced significant increase in total indols as compared with the other combinations .Meanwhile, both L.P.W. overlay and control stored for 6 months as well as desiccation stored for 6 months induced significant increase in total carbohydrates as compared with the other combinations used. On the other hand, the highest value of T.S.S. and osmotic potentiality were recorded with sorbitol (5, 10 and 15 mg/l) after 6 months but the lowest value were recorded with 1.5 mg/l coumarin after 18 months. However, significant increase of polyphenol oxidase occurred when 10 g/l PEG was used and stored for 18 months as compared to the other combinations. Meanwhile, the highest peroxidase was recorded when the explant stored under room temperature for 18 months. These results are in coordination with the findings of **Ahmed (1997)** who detected high indoles and phenoles in the treated *Bouginvillea sp. Jasminum humile* and *Malvaviscus arboreus* plants with cycocell.

Table (7): Effect of germplasm conservation treatments and storage durations on some chemical characters of *Acacia salicina* explants.

Table (7-A): Effect of germplasm conservation treatments

Treatments	Chlorophyll A Mg/100g F.W	Chlorophyll B Mg/100g F.W	Carotenoids Mg/100g F.W	Total Phenols Mg/100g F.W	Total Indole Mg/100g F.W	Total Carbohydrates Mg/100g F.W	T.S.S %	Osmotic Potentiality Atm.	PPO U/ml.min	POD U/ml.min
Control	21.55	10..99	35.70	8.77	281.45	21.53	12.49	10.75	7.08	213.37
Low temperature 4°C	10..29	4.47	12.71	0.80	39.64	11.58	5.96	4.85	6.04	9.56
P.E.G. 10 g/l	26.28	14.61	39.95	7.65	170.02	19.94	15.62	13.92	22.37	27.54
P.E.G. 15 g/l	64.48	21.38	68.50	10.10	297.99	20.28	12.87	10.99	13.12	32.03
P.E.G. 20 g/l	36.04	14.86	45.78	3.43	209.88	17.45	12.47	10.67	19.76	190.67
Desiccation	33..95	19.44	51.44	1.31	112.59	26.18	13.82	12.35	19.73	238.78
L.P.W. Soakng	27.86	11.73	29.40	3.28	118.53	16.47	7.84	11.49	12.79	112.27
L.P.W Overlaying	73.15	46.08	67.69	1.47	89.94	23.22	12.22	6.28	20.52	73.34
Coumarin 0.5mg/l	43.88	11.82	50.19	8.93	265.55	22.07	10.00	10.48	10.14	40.66
Coumarin 1.0mg/l	43..33	19.11	53.99	5.93	207.95	20.95	10.40	8.38	18.24	68.47
Coumarin 1.5mg/l	42..53	19.88	59.28	8.71	360.99	19.66	11.87	8.94	7.09	53.72
Sorbitol 5g /l	40.70	25.42	60.70	7.87	159.32	17.85	15.84	10.58	5.24	84.42
Sorbitol 10g /l	41.06	22.86	49.73	4.18	173.49	20.25	13.31	14.59	12.95	42.21
Sorbitol 15g /l	40..38	21.76	47.49	5.34	161.13	24.32	4.91	12.22	3.22	65.25
Encapsulation	9.55	9.18	10.27	0.35	154.59	8.10	2.5	4.29	0.94	6.25
L . S . D. 5 %	8.44	4.98	5.72	1.61	16.67	2.9	2.5	2.75	---	---

Table (7-B): Effect of storage duration of germplasm preservation

Treatments	Chlorophyll A Mg/100g f.w.	Chlorophyll B Mg/100g f.w	Carotenoids Mg/100g f.w	Total Phenols Mg/100g f.w	Total Indols Mg/100g f.w	Total Carbohydrates Mg/100g f.w	T.S.S %	Osmotic Potentiality Atm.	PPO U/ml.min	POD U/ml.min
6 months	99.24	47.82	113.90	12.72	113.71	26.92	12.91	12.15	7.58	49.13
12 months	53.30	35.76	70.98	8.97	137.48	15.19	13.60	12.08	7.07	39.56
18 months	34.38	14.82	45.91	8.12	108.51	17.98	11.20	10.36	6.41	24.72
L.S.D 5 %	4.35	22.37	4.22	**0.82**	4.61	**1.18**	**0.85**	**1.38**	---	---

Table (7-C1): Effect of interaction between preservation treatments and storage durations.

Treatments	Chlorophyll - A			Chlorophyll - B			Carotenoids			Total Phenols			Total Indols		
	6	12	18	6	12	18	6	12	18	6	12	18	6	12	18
Control	27.07	12.66	24.91	14.54	12.61	5.83	48.22	28.41	30.48	4.34	2.73	19.24	77.38	177.56	589.42
Cold	21.78	9.08	-	7.50	5.90	-	20.17	17.96	-	1.84	0.55	-	57.31	61.61	-
P.E.G 10 g/l	14.11	48.79	15.95	8.42	28.95	6.45	29.53	45.39	44.95	11.37	4.03	7.54	89.57	164.87	255.61
P.E.G 15 g/l	16.70	146.06	30.68	14.89	39.80	9.45	48.04	121.10	36.35	15.16	4.47	10.67	260.55	198.66	434.77
P.E.G 20 g/l	25.80	62.18	20.13	15.72	21.54	7.31	34.19	74.12	29.02	1.35	3.80	5.13	97.68	283.30	248.66
Desiccation	40.15	13.95	47.75	16.25	22.40	19.66	77.11	28.96	48.23	0.74	1.47	1.71	149.02	91.54	97.23
L.P.W. Soaking	20.85	27.24	35.49	8.60	14.76	11.84	24.97	28.45	34.78	5.31	4.11	0.43	62.80	207.93	84.87
L.P.W. overlay	145.51	63.06	10.86	99.68	31.89	6.66	114.15	75.34	13.57	1.03	0.89	2.48	68.40	116.58	84.86
Coumarin 0.5mg/l	49.89	35.53	49.21	21.86	4.63	8.95	70.80	46.34	33.44	2.29	5.15	12.35	122.99	246.10	427.58
Coumarin 1.0mg/l	48.90	44.07	37.02	20.02	23.26	14.04	77.39	44.65	39.92	7.21	2.05	8.55	73.72	181.22	368.91
Coumarin 1.5mg/l	44.53	31.29	51.76	23.64	18.22	17.80	84.50	44.16	49.19	4.57	8.78	12.79	79.75	456.12	547.10
Sorbitol 5g/l	38.95	47.19	35.97	34.67	34.99	6.60	88.44	51.81	41.87	5.09	5.06	13.45	89.26	212.44	176.28
Sorbitol 10 g/l	4.95	49.74	68.51	4.10	47.71	16.77	39.63	46.06	63.49	2.86	5.76	3.92	126.94	340.37	53.16
Sorbitol 15g/l	39.14	56.80	25.20	27.10	27.36	10.82	43.46	70.23	28.77	4.40	8.16	3.45	134.87	135.97	212.56
Encapsulation	28.65	-	-	27.54	-	-	30.79	-	-	1.04	-	-	463.76	-	-
L.S.D 5 %	14.63			8.63			9.91			2.79			28.87		

Table (7-C2): Effect of interaction between preservation treatments and storage durations.

Treatments	Total Carbohydrates			T.S.S			Osmotic potentiality			Poly phenol oxydase			Peroxydase		
	6	12	18	6	12	18	6	12	18	6	12	18	6	12	18
Control	18.41	6.90	39.29	12.27	8.33	16.87	10.39	6.73	15.13	6.39	4.66	10.19	590.36	24.02	25.75
Cold	26.27	8.47	-	10.33	7.53	-	8.57	5.99	-	15.86	2.28	-	20.23	8.45	-
P.E.G 10 g/l	23.31	21.23	15.27	16.80	13.73	16.33	15.18	11.84	14.74	6.53	17.18	43.41	23.57	17.81	44.24
P.E.G 15 g/l	23.21	11.72	25.89	13.73	11.80	13.07	11.85	9.95	11.17	4.89	17.07	17.41	21.45	28.28	46.36
P.E.G 20 g/l	21.25	9.59	21.51	13.93	12.40	11.07	12.16	10.55	9.28	8.27	12.01	39.00	339.83	65.44	166.75
Desiccation	43.69	19.79	15.07	18.20	8.00	15.27	17.10	6.53	13.44	32.32	17.12	9.75	500.50	134.36	81.49
L.P.W. Soaking	16.14	17.57	15.68	12.33	15.73	11.87	10.52	13.94	10.03	8.41	9.68	20.29	171.30	39.26	126.25
L.P.W. overlay	21.40	12.89	35.36	7.93	8.13	7.47	6.34	6.54	5.96	9.62	45.03	6.92	50.57	153.58	15.87
Coumarin 0.5mg/l	16.55	17.30	32.35	15.53	9.60	11.53	13.89	7.87	9.69	4.76	5.74	19.93	6 14	27.00	88.84
Coumarin 1.0mg/l	15.57	16.76	30.53	8.53	6.80	14.67	6.95	5.40	12.79	4.81	28.68	21.22	63.02	39.46	102.93
Coumarin 1.5mg/l	14.84	21.73	22.41	11.53	15.47	4.20	9.72	13.79	3.30	8.15	6.7	6.42	96.19	21.78	43.19
Sorbitol 5g/l	25.32	15.17	13.07	20.60	6.20	8.80	19.70	4.90	7.13	4.51	3.21	8.00	40.19	78.68	134.25
Sorbitol 10 g/l	22.41	21.23	17.10	20.33	9.53	17.67	19.70	7.82	16.23	25.18	3.81	9.85	27.19	55.34	44.12
Sorbitol 15g/l	37.63	21.67	13.65	22.87	8.07	9.0	22.85	6.48	7.34	2.49	3.92	3.26	81.78	67.61	46.36
Encapsulation	24.29	-	-	14.73	-	-	12.86	-	-	2.81	-	-	18.76	-	-
L.S.D 5 %	5.01			4.32			2.79			------			------		

3- *Rosa polyantha*

3-a–Effect of germplasm preservation treatments on physical characters.

Data in **Table (9)** reflect the effect of germplasm preservation treatments and storage durations on physical characters of *Rosa polyantha* explants. It is clear from **Table (9- A)** that coumarin at 0.5 mg/l and storage under low temperature (4°C) in the dark conditions showed the highest survival percentage followed by 1.0 mg coumarin treatment then 15 g/l sorbitol in comparison to the other used treatments. On the contrary, storage of the explants under low temperature (4°C) in the dark condition treatments succeeded significantly in improving all physical properties as induced better number of shoot and shoots length as well as moisture content of shoots percentage in relation to the other treatments. However, significant increase of number of leaves/ shoots values occurred when coumarin at 1.0 mg/l followed by coumarin at 0.5 mg/l then L.P.W. soaking as compared to the other used treatments and control in a descending order. However, **Table (9-B)** indicates that all parameters under study (survival percentage, number of shoots, shoot length and moisture contents of shoots) decreased with increasing storage duration from 6 to 12 months then 18 months respectively. On the other hand, both storage duration 6 and 12 months failed to induce any statistical differences when number of leaves/shoot was considered. Meanwhile, the interaction between germplasm preservation treatments and storage durations in **Table (9-C)** shows that all treatments were terminated due to the loss of survival percentage of storage duration after 12 and 18 months except using coumarin at either 0.5 or 1.0 mg/l and sorbitol at 15 g/l as well as preservation under low temperature (4°C) in the dark condition after 12 months. In addition storage under low temperature (4°C) in the dark

condition treatment after 6 and 12 months succeeded in maintaining acceptable number of shoots followed by both desiccation and sorbitol at 10 g/l after 6 months as compared to the other combinations in this respect. However, significant increase in shoot length values occurred when storage of the explants under low temperature (4°C) in the dark condition treatment after 6, 12 and 18 months as compared with the other used combinations and the control. Meanwhile, using sorbitol at 15 g/l stored for 12 months as well as 1.0 mg/l coumarin stored for 18 months induced highly significant increase in number of leave/shoot followed by using coumarin at 1.0 mg/l and sorbitol at (0.5 and 1.0 mg/l) 6 months then coumarin at 1.5 mg/l after 6 months as compared to the other combinations in this respect. Furthermore, storage the explants under low temperature (4°C) in the dark condition treatment after 6 months as well as L.P.W. overlay were statistically increase moisture content of shoot percentage in comparison with other used combinations and the control .

In general storage the explants under low temperature (4°C) in the dark condition treatment for 6 and 12 months succeeded in improving most of physical properties. These results are in agreement with the findings of **Wanass *et al.* (1986)** they stated the storing of pear genotypes at (4°C) for 6, 12 and 18 months is preferred in increasing storage period compared with 28°C storage .Also, with the findings of **Arora and Bhojwani (1989)** they found that shoot cultures of (*Saussurea laoa*) Asteraceae were stored at 5°C in the dark for 6 months or more showed increase in multiplication rate than the shoots maintained under room temperature. Beside, **Weijia *et al.* (1999)** on Williams banana plants. They found that culturing of banana explants on MS medium supplemented with paclobutrazol and CCC stimulated increase in plant height.

Table (9): Effect of germplasm conservation treatments and storage durations on some physical characters of *Rosa polyantha* explants *vitro* after storage duration

Table (9-A): Effect of germplasm conservation treatments.

Treatments	Survival%	Callusing %	Shoot No.	Shoot length cm	Leaves No.	Shoots M.C.
Control	53.33	66.67	1.31	0.47	3.72	47.12
Low temperature 4°C	86.67	94.33	2.45	2.64	5.36	86.16
P.E.G. 10 g/l	21.33	33.33	0.46	0.13	2.01	22.12
P.E.G. 15 g/l	28.00	33.33	0.41	0.23	3.30	27.33
P.E.G. 20 g/l	0.00	0.00	0.00	0.00	0.00	0.00
Desiccation	26.67	33.33	0.63	0.17	2.04	16.54
L.P.W. Soaking	46.67	61.00	0.68	0.28	7.74	55.25
L.P.W Overlaying	22.22	24.44	0.56	0.40	2.09	30.54
Coumarin 0.5 mg/l	88.00	4.00	1.01	1.10	9.83	64.84
Coumarin 1.0 mg/l	84.00	23.00	1.03	1.25	10.77	64.23
Coumarin 1.5 mg/l	46.67	66.67	0.99	0.83	6.28	44.12
Sorbitol 5g /l	44.00	33.33	0.83	0.75	5.61	42.02
Sorbitol 10g /l	49.33	33.33	0.98	0.72	5.98	44.09
Sorbitol 15g /l	61.33	33.33	0.92	0.56	5.88	41.85
Encapsulation	10.67	8.00	0.00	0.00	0.00	27.04
L.S.D. at 5%	2.87	2.61	0.07	0.09	0.45	1.79

Table (9-b): Effect of storage duration.

Treatments	Survival %	Callusing %	Shoot No.	Shoot length cm	Leaves No.	Shoot M.C.
6 months	77.51	75.16	1.33	0.77	5.97	67.17
12 months	41.60	27.20	0.88	0.75	5.98	41.53
18 months	14.67	7.27	0.25	0.38	2.17	13.96
L.S.D at 5%	0.57	0.52	0.02	0.02	0.09	0.59

Table (9-C1): Effect of interaction between conservation treatments and storage durations.

Treatments	Survival %			Callusing %			Shoot Number			Shoot Length cm			Leaves Number		
	6	12	18	6	12	18	6	12	18	6	12	18	6	12	18
Control	96	64	0	100	100	0	1.40	2.53	0	0.76	0.64	0.00	5.75	5.40	0.00
Cold	96	96	68	83	100	100	2.83	2.77	1.76	2.29	2.95	2.67	4.52	5.27	6.30
P.E.G 10 g/l	64	0	0	100	0	0	1.38	0	0	0.40	0.00	0.00	6.02	0.00	0.00
P.E.G 15 g/l	84	0	0	100	0	0	1.23	0	0	0.68	0.00	0.00	9.90	0.00	0.00
P.E.G 20 g/l	0	0	0	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00
Desiccation	79.99	0	0	100	0	0	1.90	0	0	0.51	0.00	0.00	6.12	0.00	0.00
L.P.W. Soaking	92	48	0	83	100	0	1.04	1.0	0	0.84	0.00	0.00	7.76	15.45	0.00
L.P.W. overlay	66.66	0	0	73.33	0	0	1.67	0	0	1.19	0.00	0.00	6.28	0.00	0.00
Coumarin 0.5mg/l	92	96	76	12	0	0	1.0	1.04	1.0	0.83	0.95	1.51	8.97	8.11	12.40
Coumarin 1.0mg/l	84	92	76	52	8	9	1.10	1.0	1.0	0.82	1.39	1.52	7.05	11.46	13.79
Coumarin 1.5mg/l	96	44	0	100	100	0	1.65	1.33	0	1.00	1.48	0.00	10.30	8.55	0.00
Sorbitol 5g/l	92	40	0	100	0	0	1.41	1.07	0	0.99	1.26	0.00	5.63	11.20	0.00
Sorbitol 10 g/l	96	52	0	100	0	0	1.79	1.15	0	0.60	1.55	0.00	6.89	11.05	0.00
Sorbitol 15g/l	92	92	0	100	0	0	1.52	1.25	0	0.69	0.99	0.00	4.43	13.22	0.00
Encapsulation	32	0	0	24	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00
L.S.D 5 %	8.61			0.21			0.27			1.34			5.36		

Table (9-C2): Effect of interaction between preservation treatments and storage durations.

Treatments	*Shoot M.C.*		
	6	12	18
Control	74.97	66.38	0.00
Cold	91.63	86.03	80.83
P.E.G 10 g/l	66.36	0.00	0.00
P.E.G 15 g/l	82.00	0.00	0.00
P.E.G 20 g/l	0.00	0.00	0.00
Desiccation	49.63	0.00	0.00
L.P.W. Soaking	83.48	82.27	0.00
L.P.W. overlay	91.61	0.00	0.00
Coumarin 0.5mg/l	70.11	57.17	67.25
Coumarin 1.0mg/l	66.77	64.67	61.26
Coumarin 1.5mg/l	70.36	61.99	0.00
Sorbitol 5g/l	57.45	68.60	0.00
Sorbitol 10 g/l	64.69	67.57	0.00
Sorbitol 15g/l	57.32	68.23	0.00
Encapsulation	81.11	0.00	0.00
L.S.D at 5%	5.36		

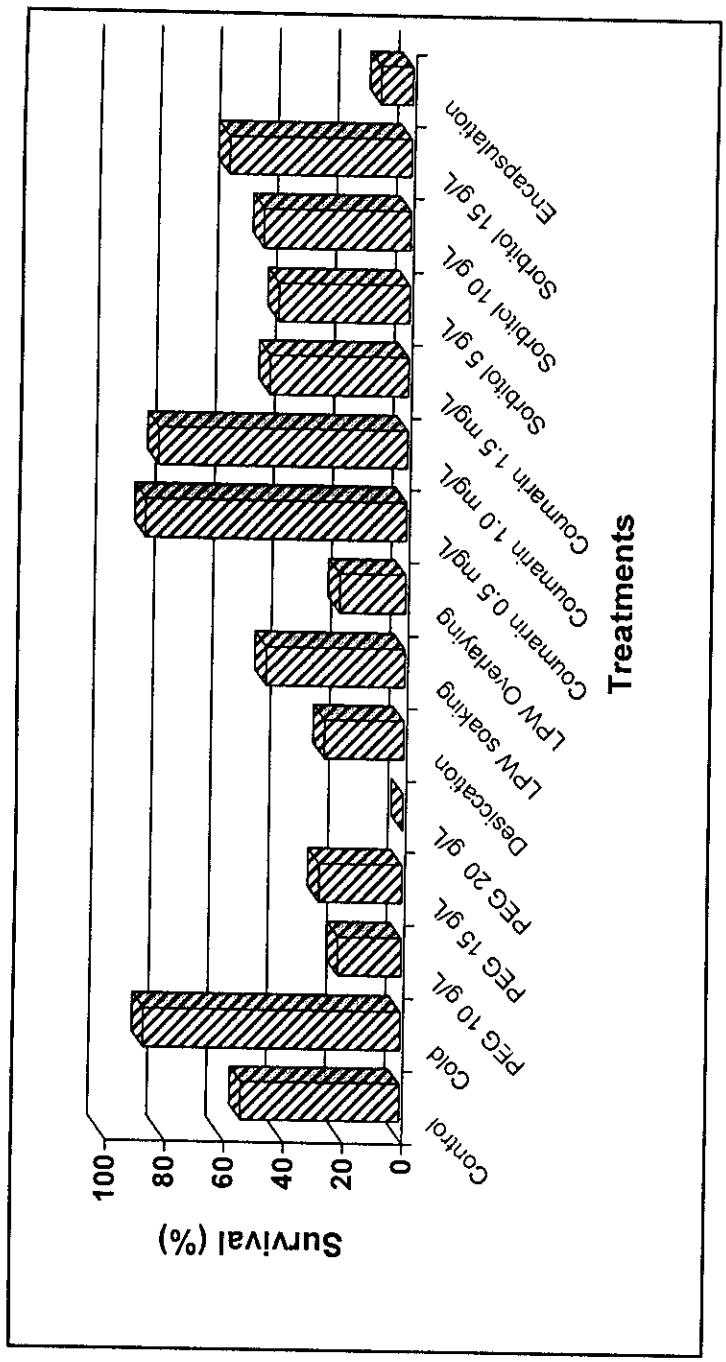

Fig. (3): Effect of germplasm preservation treatments on survival rate of *Rosa polyantha* explants *in vitro* after storage duration

3-b-Effect of germplasm preservation treatments on chemical characters.

Table (10) shows the effect of germplasm preservation treatments and storage duration on chemical characters of *Rosa polyantha* explants. It is clear from **Table (10–A)** that using coumarin at 0.5 mg/l gave significantly the highest chlorophyll A followed by coumarin at 1.0 mg/l then sorbitol at 10 g/l as compared to the other used treatments and the control. Meanwhile, sorbitol at 10 g/l showed the highest chlorophyll B followed by coumarin at 0.5 and 1.0 mg/l among the other treatments. Besides, coumarin at 1.0 mg/l gave significantly the highest carotenoids followed by coumarin at 0.5 mg/l then sorbitol at 10 g/l compared to the other used treatments and the control. However, significant increase of total phenols, total indoles and total carbohydrates as well as T.S.S. occurred when both coumarin at 0.5 and 1.0 mg/l were used. Furthermore, using coumarin at 0.5 and 1.0 mg/l as well as desiccation and PEG at 15 g/l caused the highest effect in increasing osmotic potentiality as compared with the other used treatments and the control. On the other hand, no significant differences were noticed between different treatments and the control when polyphenol oxidase and peroxidase were concerned. It is clear from **Table (10–B)** that all chemical characters under study were decreased by increasing storage duration from 6 to 12 and 18 months in a descending order. On the other hand, statistical differences were lacked when polyphenol oxidase and peroxidase were considered .It is obvious from **Table (10–C)** that sorbitol at 10 g/l after 6 months increased chlorophyll A and B statistically. However, significant increase in carotenoids were recorded when sorbitol at 10 g/l stored for 6 months as well as coumarin at 0.5 mg/l

stored for 12 months were used. Meanwhile, using PEG at 10 g/l and sorbitol at 5g/l stored for 6 months as well as coumarin at 0.5 mg/l stored for 18 months increased total phenols as compared to the other combinations and the control. Furthermore, using sorbitol at 15 g/l and desiccation stored for 6 months induced significant increase in total indoles foliowed by sorbitol at 10 g/l stored for 6 months as well as coumarin at 0.5 mg/l as compared with the other combinations. On the contrast, using desiccation and PEG at 10 g/l after 6 months as well as sorbitol stored for 6 months encouraged higher total carbohydrates followed by 5 g/l sorbitol stored for 6 months as compared to the other combinations .Meanwhile, significant increase in T.S.S. and osmotic potentiality values occurred when PEG at 15 g/l stored for 6 months was used. On the other hand, combinations of germplasm conservation treatments and storage durations failed to induce any statistical difference among them when polyphenol oxidase and peroxidase were concerned. These results are in harmony with the findings of **Chaves *et al.* (1999)** They found that storing of celery plant (cv. Golden Boy) at 0°C for 28 days resulted in reducing of chlorophyll contents rapidly. Also, with the findings of **Ahmed (1997)** he recommended using of 6000 mg/l CCC for increasing both phenols and indoles contents in *Bougainvilla sp* and 4000 mg/l CCC for *Jasmunium humile* as well as 5000 mg/l for *Malvaviscus arboreus.*

Table (10): Effect of germplasm conservation treatments and storage durations on some chemical characters of *Rosa polyantha* explants.

Table (10-A): Effect of germplasm conservation treatments.

Treatments	Chlorophyll A	Chlorophyll B	Carotenoids	Total Phenols	Total Indols	Total Carbohydrate	T.S.S	Osmotic potentiality	Poly phenol oxydase	Peroxydase
Control	77.92	33.26	96.03	9.71	237.96	13.03	9.27	7.92	0.78	8.43
Low temperature 4°C	19.20	5.36	19.46	3.32	100.98	12.29	8.47	6.96	1.98	7.83
P.E.G. 10 g/l	14.08	5.10	22.51	9.57	129.74	14.47	7.31	7.05	1.08	11.28
P.E.G. 15 g/l	15.75	4.93	15.86	7.38	115.67	12.27	8.44	8.76	0.21	0.82
P.E.G. 20 g/l	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Desiccation	34.57	28.68	98.33	7.86	282.36	15.40	8.07	8.46	0.34	3.96
L.P.W. Soaking	113.14	50.21	113.16	6.58	194.19	6.07	3.76	2.97	6.98	43.14
L.P.W Overlaying	46.84	37.94	34.20	6.00	39.90	4.28	1.78	1.40	0.62	34.14
Coumarin 0.5mg/l	217.80	63.31	194.07	22.38	411.82	29.47	9.66	8.09	1.63	2.30
Coumarin 1.0mg/l	197.78	62.12	240.69	24.32	529.01	29.51	10.67	8.88	0.78	6.14
Coumarin 1.5mg/l	90.60	34.24	83.14	14.14	203.95	18.87	7.36	6.18	0.31	0.93
Sorbitol 5g /l	90.84	32.20	113.22	13.83	306.48	21.17	3.71	2.93	0.37	6.26
Sorbitol 10g /l	137.29	76.77	145.60	13.43	387.13	21.47	3.47	2.74	0.90	3.47
Sorbitol 15g /l	102.08	30.03	82.86	14.32	407.96	14.87	3.53	2.80	3.00	4.67
Encapsulation	6.43	2.25	9.85	4.29	88.21	10.98	5.82	5.29	2.65	2.71
L.S.D. at 5%	13.36	7.31	24.72	2.25	14.61	2.29	1.25	1.48	-----	-----

Table (10-b): Effect of storage duration of germplasm conservation.

Treatments	Chlorophyll A	Chlorophyll B	Carotenoids	Total Phenols	Total Indols	Total Carbohydrates	T.S.S	Osmotic potentiality	Poly phenol oxydase	Peroxydase
6 months	132.62	53.12	146.69	17.34	394.88	30.04	11.37	10.42	2.76	10.52
12 months	77.94	36.03	88.44	11.77	217.93	11.80	6.15	5.23	2.97	4.12
18 months	24.70	6.53	21.07	4.71	76.66	5.38	3.15	2.83	3.11	12.58
L.S.D 5 %	5.98	3.27	11.06	1.00	6.54	1.03	0.56	0.67	------	-------

Table (10-C1): Effect of interaction between conservation treatments and storage durations.

Treatments	Chlorophyll - A			Chlorophyll - B			Carotenoids			Total Phenols			Total Indols		
	6	12	18	6	12	18	6	12	18	6	12	18	6	12	18
Control	186.73	47.04	0.00	65.11	34.67	0.00	203.74	84.37	0.00	7.52	21.60	0.00	284.34	429.53	0.00
Cold	1.03	16.58	39.99	3.18	3.14	9.77	4.35	23.27	30.77	1.26	5.60	3.10	89.67	99.87	113.41
P.E.G 10 g/l	42.24	0.00	0.00	15.30	0.00	0.00	67.54	0.00	0.00	28.72	0.00	0.00	389.22	0.00	0.00
P.E.G 15 g/l	47.24	0.00	0.00	14.80	0.00	0.00	47.59	0.00	0.00	22.13	0.00	0.00	347.02	0.00	0.00
P.E.G 20 g/l	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Desiccation	103.72	0.00	0.00	86.03	0.00	0.00	294.99	0.00	0.00	23.58	0.00	0.00	847.08	0.00	0.00
L.P.W. Soaking	113.77	225.64	0.00	80.71	69.92	0.00	167.87	171.59	0.00	0.50	19.25	0.00	135.05	447.52	0.00
L.P.W. overlay	140.51	0.00	0.00	113.82	0.00	0.00	102.60	0.00	0.00	17.99	0.00	0.00	119.70	0.00	0.00
Coumarin 0.5mg/l	282.10	234.54	136.75	64.62	95.99	29.33	289.27	191.61	101.32	22.43	22.21	22.50	263.84	459.29	512.34
Coumarin 1.0mg/l	182.22	235.35	175.78	66.91	78.63	40.81	197.84	358.23	165.99	22.83	23.01	27.11	752.11	328.79	506.12
Coumarin 1.5mg/l	35.37	236.42	0.00	17.94	84.79	0.00	49.11	200.31	0.00	20.66	21.77	0.00	248.78	363.06	0.00
Sorbitol 5g/l	225.80	46.72	0.00	72.21	24.40	0.00	220.23	119.43	0.00	26.70	14.79	0.00	584.30	335.13	0.00
Sorbitol 10 g/l	351.39	60.48	0.00	123.35	106.97	0.00	366.16	70.64	0.00	23.46	16.83	0.00	734.55	426.84	0.00
Sorbitol 15g/l	251.85	54.38	0.00	60.12	29.98	0.00	153.43	95.16	0.00	23.49	19.47	0.00	856.91	366.96	0.00
Encapsulation	19.30	0.00	0.00	6.75	0.00	0.00	29.54	0.00	0.00	12.88	0.00	0.00	264.64	0.00	0.00
L.S.D 5 %	23.15			12.67			42.82			3.89			25.31		

Table (10-C2): Effect of interaction between conservation treatments and storage durations.

Treatments	Total Carbohydrates			T.S.S			Osmotic potentiality			Poly phenol oxydase			Peroxydase		
	6	12	18	6	12	18	6	12	18	6	12	18	6	12	18
Control	16.19	22.88	0.00	13.60	14.20	0.00	11.69	12.05	0.00	1.60	0.73	0	22.11	3.18	0
Cold	22.71	4.67	9.49	11.33	9.20	4.87	9.51	7.54	3.85	0.76	2.08	3.11	2.33	8.99	12.17
P.E.G 10 g/l	43.41	0.00	0.00	21.93	0.00	0.00	21.14	0.00	0.00	3.25	0.00	0	33.85	0.00	0
P.E.G 15 g/l	36.82	0.00	0.00	25.33	0.00	0.00	26.28	0.00	0.00	0.63	0.00	0	2.45	0.00	0
P.E.G 20 g/l	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0	0.00	0.00	0
Desiccation	46.19	0.00	0.00	24.20	0.00	0.00	25.37	0.00	0.00	1.03	0.00	0	11.89	0.00	0
L.P.W. Soaking	18.21	0.00	0.00	5.40	5.87	0.00	4.27	4.63	0.00	19.57	1.37	0	12.25	4.24	112.92
L.P.W. overlay	12.84	0.00	0.00	5.33	0.00	0.00	4.21	0.00	0.00	1.86	0.00	0	36.20	2.63	63.60
Coumarin 0.5mg/l	34.96	25.59	27.86	4.83	10.53	13.60	3.83	8.73	11.71	3.21	0.54	1.14	3.79	3.11	0
Coumarin 1.0mg/l	36.48	26.67	25.37	9.53	11.73	10.73	7.80	9.89	8.93	1.05	0.27	1.02	9.73	8.69	0
Coumarin 1.5mg/l	35.36	21.26	0.00	8.40	13.67	0.00	6.77	11.76	0.00	0.73	0.19	0	2.80	0.00	0
Sorbitol 5g/l	41.22	22.31	0.00	4.93	6.20	0.00	3.90	4.90	0.00	0.20	0.91	0	2.11	16.65	0
Sorbitol 10 g/l	43.35	21.06	0.00	4.73	5.67	0.00	3.74	4.48	0.00	1.95	0.76	0	3.99	6.44	0
Sorbitol 15g/l	24.00	20.60	0.00	7.47	3.13	0.00	5.95	2.45	0.00	1.71	7.28	0	6.15	7.85	0
Encapsulation	32.93	0.00	0.00	17.47	0.00	0.00	15.87	0.00	0.00	7.95	0.00	0	8.14	0.00	0
L.S.D 5 %	3.98			2.17			2.55			--------			--------		

3-C-Effect of germplasm preservation treatments on viability percentage on growth characters of regenerated plantlets.

Table (11) showed the effect of germplasm treatments and storage duration on viability percentage on growth characters of regenerated *Rosa polyantha* explants. It is clear from **Table (11-A)** that using coumarin at 0.5 and 1.0 mg/l as well as low temperature (4°C) induced significant increase in viability percentage followed by using sorbitol at 10 and 15 g/l as well as coumarin at 1.5 mg/l then L.P.W. soaking as compared to the other used treatments and the control. Meanwhile, storing under low temperature (4°C) significantly increased number of shoots followed by using coumarin at 0.5 and 1.0 mg/l among the other treatments. However, significant increase of shoot length and number of leaves shoot occurred when coumarin at 1.0 mg/l was used. A glance to **Table (11-B)** it is oblivious that growth characters of regenerated explants under study were decreased by increasing storage duration from 6 to 12 and 18 months in a descending order. However, the interaction between germplasm preservation treatments and storage durations it appears from **Table (11–C)** that sorbitol at 5 and 15 g/l and storage of the explants under low temperature (4°C) in the dark condition for 6 months as well as coumarin at 1.5 mg/l stored for 12 months and coumarin at 0.5 mg/l stored for 18 months encouraged significant increase in viability percentage in comparison to the other combinations Meanwhile, storage of the explants under low temperature (4°C) in the dark condition stored for 12 months induced significant increase in number of shoots followed by 10 g/l sorbitol stored for 12 months then 1.5 mg/l coumarin stored for 12 months as compared to the other combinations. However, significant increase of shoot length and number of leaves /shoot values occurred when coumarin at 1.5 g/l was used stored for 12 months as compared to the other combinations used and the control.

These results assured the findings of **Startsky (1986)** he found that were exposing of taro to 3 and 25°C showed very healthy shoots after 10 weeks of incubation. Also, with the findings of **Takagi and Qu (1995)** they found that all shoot tip

explants from cloves late maturing garlic (*Allium sativum* L.) which exposed to 5°C for 60 days formed large number of bulbs.

Table (11): Effect of germplasm conservation treatments and storage durations on viability and some growth characters of regenerated *in vitro Rosa polyantha* explants *in vitro* after storage duration

Table (11-A): Effect of germplasm conservation treatments.

Treatments	Viability%	Shoot No.	Shoot length cm	Leaves No.
Control	58.67	0.98	0.49	7.77
Low temperature 4°C	93.33	2.38	0.63	5.11
P.E.G. 10 g/l	26.67	0.39	0.20	1.79
P.E.G. 15 g/l	30.67	0.39	0.17	1.71
P.E.G. 20 g/l	0.00	0.00	0.00	0.00
Desiccation	24.00	0.44	0.12	1.42
L.P.W. Soaking	61.33	1.02	0.33	3.64
L.P.W Overlaying	29.33	0.48	0.15	1.92
Coumarin 0.5mg/l	92.00	1.77	0.74	7.16
Coumarin 1.0mg/l	92.00	1.56	0.82	7.19
Coumarin 1.5mg/l	64.00	1.13	0.64	4.95
Sorbitol 5g /l	58.67	1.38	0.40	3.91
Sorbitol 10g /l	65.33	1.74	0.40	3.83
Sorbitol 15g /l	64.00	1.36	0.49	4.72
Encapsulation	22.67	0.46	0.15	1.58
L.S.D. at 5%	**3.20**	**0.10**	**0.05**	**0.27**

Table (11-B): Effect of storage duration.

Treatments	Survival %	Shoot No.	Shoot length cm	Leaves No.
6 months	83.47	1.41	0.54	5.54
12 months	54.93	1.30	0.44	4.10
18 months	18.13	0.38	0.16	1.70
L.S.D at 5%	0.64	0.02	0.01	0.05

Table (11-C): Effect of inter action between germplasm conservation treatments and storage durations.

Treatments	Viability %			Shoot number			Shoot length cm			Leaves Number		
	6	12	18	6	12	18	6	12	18	6	12	18
Control	92.00	84.00	0.00	1.16	1.78	0.00	0.36	1.13	0.00	5.07	9.14	9.10
Cold	100.00	96.00	84.00	1.56	3.38	2.20	0.53	0.63	0.74	7.40	7.92	0.00
P.E.G 10 g/l	80.00	0.00	0.00	1.16	0.00	0.00	0.61	0.00	0.00	5.37	0.00	0.00
P.E.G 15 g/l	92.00	0.00	0.00	1.16	0.00	0.00	0.52	0.00	0.00	5.13	0.00	0.00
P.E.G 20 g/l	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Desiccation	72.00	0.00	0.00	1.31	0.00	0.00	0.37	0.00	0.00	4.26	0.00	0.00
L.P.W. Soaking	88.00	96.00	0.00	1.64	1.43	0.00	0.54	0.45	0.00	5.55	5.38	0.00
L.P.W. overlay	88.00	0.00	0.00	1.43	0.00	0.00	0.44	0.00	0.00	5.77	0.00	0.00
Coumarin 0.5mg/l	92.00	84.00	100.00	1.49	2.07	1.76	0.57	0.72	0.92	5.95	7.01	8.52
Coumarin 1.0mg/l	92.00	96.00	88.00	1.23	1.71	1.73	0.88	0.76	0.81	6.39	7.29	7.88
Coumarin 1.5mg/l	92.00	100.00	0.00	1.08	2.32	0.00	0.68	1.25	0.00	4.69	10.17	0.00
Sorbitol 5g/l	100.00	76.00	0.00	2.28	1.85	0.00	0.63	0.55	0.00	7.34	4.39	0.00
Sorbitol 10 g/l	100.00	96.00	0.00	2.28	2.95	0.00	0.64	0.54	0.00	6.53	4.97	0.00
Sorbitol 15g/l	96.00	96.00	0.00	2.03	2.04	0.00	0.92	0.56	0.00	8.87	5.30	0.00
Encapsulation	68.00	0.00	0.00	1.37	0.00	0.00	0.46	0.00	0.00	4.75	0.00	0.00
L.S.D at 5%	9.60			0.30			0.14			0.80		

Cryopreservation treatments:

2-1-Effect of cryopreservation treatments on physical characters

Data presented in **Table (12)** revealed that the survival rate were affected with various treatments of the cryopreservation pretreatments, in order to test this applicability of the technique to wide range of materials from *in vitro* culture, the experiments have been carried out with various types *Ceratonia siliqua Rosa polyantha and Acacia salicina. R. polyantha* explants have no responding to any pretreatment. In this concern, **Lambardi *et al.* (2000)** found that on olive (*Olea europaea*) and horsechestnut (*Aesculus hippocastanum*) were exposed to the vetrification solution (PVS2) at 0°C for 60 and 90 min, respectively. The explants were directly immersed in LN for at least one hour, while isolated somatic embryos of both olive and horsechestnut never showed recovery after cryopreservation. All the explants which soaked directly in liquid nitrogen without any pretreatment died. Moreover, on dehydration treatment under various unsaturated salts resulted 13.33% under $CaCl_2$ for *A. salicina* but increase but increase to 20% for *C. siliqua*. Moreover, under silica gel the survival was decreased to 6.66 % for each one. In this concern, **Engeleman (1995)** reported that on oil palms embryos were recorded desiccation to 0.3 g H_2O/g F.W. in the air flow, 65 % developed into plantlets after cryopreservation. In contrast, only 25%

embryos (0.12 g H_2O D.W.) extracted from cryopreserved dry kernels developed into plantlets. On the other hand, on vitirification the first treatment (VI) recorded increase in the value to 33.3 % for *C. siliquia* and decrease to 0 % for *Acacia salicina*. Beside that on the second treatment (V2) resulted 26.66 for *C.siliquia* and decline sharply to 6.66 % for *A. salicina*. Also, both of V3 and V4 gave the same effect of the survival rate on *A. salicina* resulted 13.33 and 0 % respectively but on *C. siliqua* increased to 20 % for each on. In this regard, **Lambardi *et al.* (2000)** observed that on poplar (*Populus spp.*), plum (*Prunus domstica*), ollve (*Olea europaea*) and horsechestnut (Aesculuc hippocastanum) were exposed to the vitrification solution (PVS2) at 0°C for 60 and 90 min, respectively. The explants were directly immersed in L.N. for at least one hour. The high percentage of shoot tip survival were obtained with *Populus alba* (90%) and Plum (47%) on olive a maximum of 15 % post- thaw survival was obtained with the application of the vitrification procedure to shoot tips from *in vitro* grow plantlets of the cv. Frantoio. The effect of vitrification solution were explain with **Panis *et al.* (2000)** the care must be taken to avoid ice crystallization during the freezing process, which other wise would cause physical damage to the tissue. The existing cryogenic strategies rely of freeze-dehydration, addition of cryoprotective substances including the recently developed plant vitrification solutions, desiccation, induced metabolic changes in tissues to make them more tolerant to cryopreservation or

combinations of these processes. However, pregrowth treatments (I), (II) resulted 6.66 for each one for *Acacia salicina* and increased to 40 % and 33.335 for *C. siliquia* explants. In this concern, **Jekkel *et al.* (1998)** found that culturing of somatic embryos of horse- chestnut (*Aesculus hippocastsnum* L.) on nutritive media containing ABA 0.75 µM, 7.5 µM and 75 µM for 4 days, followed by cryoprotictive treatment in liquid media containing 0.5 M DMSO, 0.5 M glyserol, 1.0 sucrose and then immersed in liquid nitrogen had the best embryo recovery 43%. Also, **Anadarajah and Mckersie (1990)** found that culturing of somatic embryos of *Medicago sativa* L. were cultured on ABA in the medium gave the best result of dehydration tolerance. On the encapsulation dehydration of *Acacia salicina* gave a low value for dehydration with $CaCl_2$ resulted 13.33 5 and have no recovery for dehydration with silica gel, But this value gave the best value of the encapsulation dehydration of *Ceratonia siliqua* gave 40% and 20 fore $CaCl_2$ and silica gel respectively. In this regard, **Vandenbussche (1998)** found that on *Beta vulgaris* L. colme 0 were cryopreserved using the encapsulation dehydration technique of shoot tips, survival rate of 37 % after freezing. Also, this was shown for *P. communis* L. shown by **Scottez *et al.* (1992)**, *M. domestica* Brokh by **(Niino and Sakai 1992)** and *Ribes sp.* and cultivars **(Reed and Yu 1995)**.

Table (12): Effect of some pretreatment of cryopreservation on some survival rate of *Rosa polyantha, Acacia salicina and Ceratonia siliqua in vitro* explants after soaking in liquid nitrogen

Treatments	*Rosa polyantha*	*Acacia salicina*	*Ceratonia siliqua*
Control	0	0.00	0.00
$CaCl_2$ dehydration	0	13.33	20.00
Silica gel dehydration	0	6.67	6.67
PVS1	0	0.00	33.33
PVS2	0	6.67	26.67
PVS3	0	13.33	20.00
PVS4	0	0.00	20.00
PI	0	6.67	40.00
P II	0	6.67	33.33
E / D I	0	13.33	40.00
E / D II	0	0.00	20.00
L.S.D at 5%	00	0.012	0.254

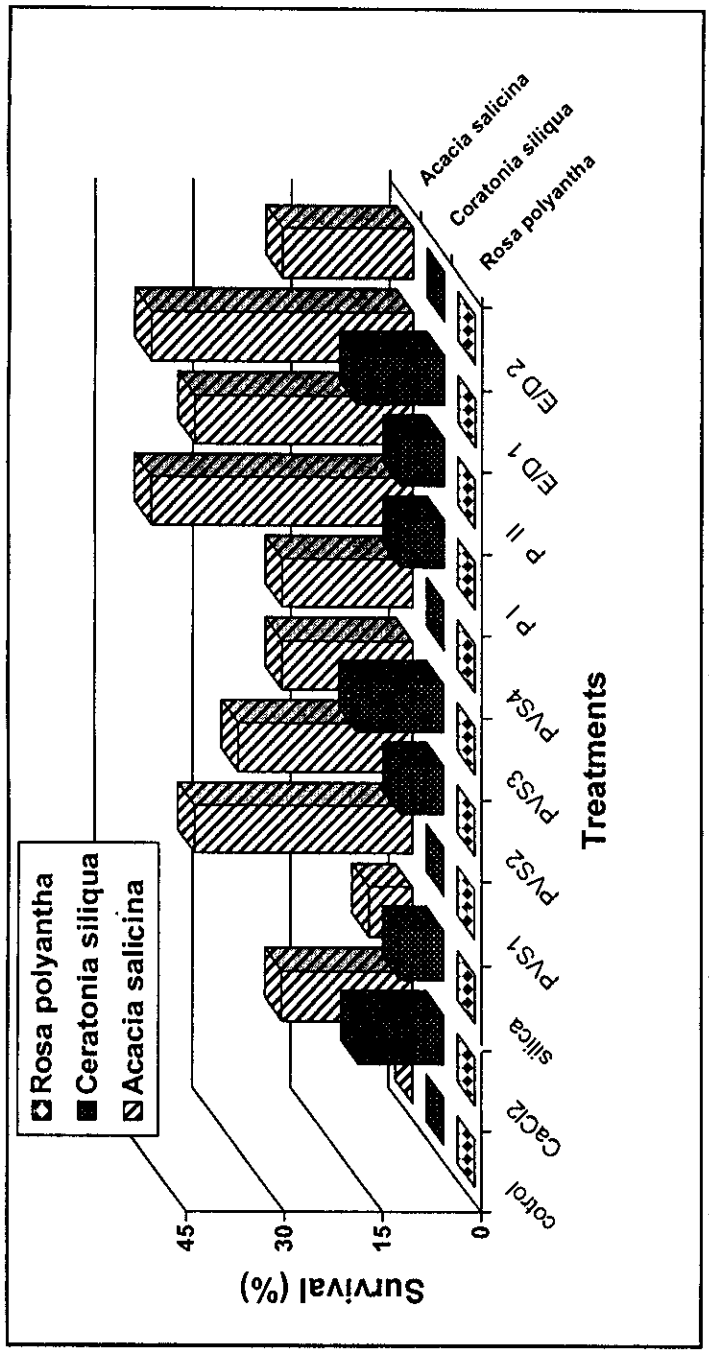

Fig. (4): Effect of cryopreservation pretreatments on survival rate of *Ceratonia siliqua, Acacia salicina and Rosa polyantha* explants *in vitro* after soaked in liquid nitrogen.

2-B. Effect of cryopreservation treatments on chemical characters

Data presented in **Table (13)** demonstrated that the total chlorophyll- A of *Acicia salicina* were influenced with pretreatment of cryopreservation of all treatments as they decreased of their endogenous chlorophyll –A except with explants exposed to cryoprotactant solutions (PVS I) and (PVS3) which resulted in 139.67 and 160.57 mg/100 g F.W. compared to 92.39 mg/100 g F.W for the control. However, on **Table (14)** showed that the chlorophyll – A resulted in 126.03 mg/100 g F.W for the *Ceratonia siliqua* explants plunged directly in liquid nitrogen without any pretreatment this value decreased to the highest value when drying conditions under $CaCl_2$ (322.68 mg/100 g F.W) was used. Moreover, silica gel induced the same effect compared to control, it recorded 156.6 mg/100 g F.W. However, all the other treatments gave a sharply decrease in their contents of chlorophyll – A of *C. siliqua* explants compared to control except with soaking in cryoprotactant solution (PVS 1) and (PVS2) which recorded 142.87 and 139.89 mg/100 g F.W respectively, but the other treatments ranged between 46.79 and 87.51 mg/100 g F.W. Furthermore, Data presented in **Table (15)** revealed that the amount of chlorophyll-A in tissues were assayed after treatments of various methods, the explant exposed to drying condition ($CaCl_2$) maximized chlorophyll-A in *R. polyantha* to the highest value of there contents (363.2 mg/100 g F.W were recorded compared to 309.64 mg/100 g F.W. The same effect was occurred in the explant cultured on MS containing 1.5 mg/l for one week which raised the endogenous chlorophyll – A from 309.64 for the control to 347.34 mg/100 g F.W for the pregrowth treatment.

Moreover, all the other treatments ranged their decrease between 38.68 for encapsulation dehydration (on silica gel 0 and 243.55 mg/100 g F.W for dehydration under silica gel

Data presented in **Table (13)** revealed that the endogenous chlorophyll-B were assayed after plunged in liquid nitrogen for *Acacia salicina* explants *in vitro* the highest amount were found 67.99 mg/100 g F.W for the explants treated in cryoprotactants solution (PVS3). Meanwhile, the lowest contents were 15.42 for the explants treated with DMSO and glycerol on medium (pregrowth II) but the explants plunged directly in liquid nitrogen without any pretreatment resulted 48.22 mg/100 g F.W. However, the data presented in **Table (14)** on *Ceratonia siliquia in vitro* explants were resulted the highest contents of chlorophyll-b were determined 161.39 mg/100 g F.W for the explants drying under $CaCl_2$ condition compared with the explants plunged in liquid nitrogen (48.6 mg/100 g F.W). Meanwhile, the lowest value were 24.39 mg/100 g F.W for the explant exposed to encapsulation dehydration on silica gel. However, desiccation under silica gel and soaking in cryoprotactant solution (PVS1 and PVS 2) enhanced increase in chlorophyll – B compared to control. Furthermore, On *Rosa polyantha* the contents of chlorophyll- B shown in **Table (15)** the highest value were recorded for the explants exposed to drying condition with $CaCl_2$ it (158.27 mg/100 g F.W) compared to control (157.19 mg/100 g F.W.) However, the lowest value was observed for the explants treated with encapsulation dehydration under silica gel. Also, all the other pretreatments induced decrease in the value compared to 157.19 mg/100 g F.W.

The results presented in **Table (13)** revealed that the effect of pretreatment of cryopreservation on total carotenoid, of *in vitro Acacia salicina* explants .The highest value was recorded in the explants which exposed to cryoprotactants solution (PVS 3) 143.73 mg/100 g F.W. Meanwhile, the lowest value observed in encapsulation dehydration under $CaCl_2$ treatment (49.26 mg/100 g F.W.) Desiccation under silica gel, Pregrowth (I) and (II) which gave a decline in their contents compared with 77.10 mg/100 g F.W for the control. Moreover, all the other pretreatment resulted a decrease in their contents compared with control it ranged between 77.15 and 119.06 mg/100 g F.W. On the other hand, on **Table (14)** the total carotenoids on *C. siliqua in vitro* explants which induced the highest amount of endogenous total carotenoids for the explants desiccated under $CaCl_2$ conditions (284.22 mg/100 g F.W) compared to 150.31 mg/100 g F.W for the control. Only soaking in cryoprotactant solution (PVS 1) increased endogenous total carotenoid (172.52- mg/100 g F.W). Meanwhile, all the other pretreatment gave a sharp decline in their value as it ranged from 62.19 to 122.01 mg/100 g F.W. Furthermore, Data presented in **Table (15)** revealed that the explants of *Rosa polyantha* dried under CaCl2 recorded 350.08 mg/100 g F.W compared to 250.64 mg/100 g F.W for the explants plunged in liquid nitrogen directly (control). Also, only desiccation under $CaCl_2$ resulted increased in their endogenous contents of total carotenoids (341.77) However, all the other pretreatment-recorded decrease in the total carotenoid compared to control (85.76 and 292.93).

Data presented in **Table (14)** revealed that the endogenous total phenols of *Ceratonia siliquia* influenced with various pretreatment of immersion in liquid nitrogen it resulted

14.11 for the control (immersion directly without treating any pretreatment) Meanwhile, the endogenous total phenol were induced the highest level when the explant exposed to drying condition ($CaCl_2$), it was 38.95 mg/100 g F.W. while the level decrease slightly to 28.09 mg/100 g F.W. for drying under silica gel, In this concern, **Berctch *et al.* (1990)** found that exposing of *Picea abies* and *Picea glauca* calli to high concentration of 1,2 propandiol appear to be toxic for axes with a 5 % moisture content (only 30% of regrowth of the control) Due to a solute concentration effect within cells of the embryonic axes as a result of dehydration. On the other hand, vitrification treatments both of (V1) and (V4) have a positive effect to increase total phenols compared with control but V2 and V3 resulted in decrease their endogenous total phenol compared with 14.11 for the control. In this concern, On imbibed *Zea mays* seeds have shown that the cryoprotectant function of 1,2 propandiol increased with the addition of sucrose. The effect as trend the explants were encapsulation / dehydration with calcium chloride (E/D1) or silica gel (E/D2) or pregrowth with coumarin (PI) 1.5 mg/l and using DMSO and glycerol gave a decrease in the endogenous total phenols. On the other hand, exposing of *Acacia salicina* explants to (P2) gave the highest level of the phenols, it was 14.6 mg/100 g F.W. compared with 11.41 mg/100 g F.W. for the control and decline sharply when exposed to (P1) , it resulted 10.35 mg/100 g F.W. Meanwhile, all the other treatments gave low values of their contents compared to control. However, on *R. polyantha* the endogenous total phenols were higher compared with the other types, on control (immersion without pretreatment) gave 53.96 mg/100 g F.W. the dehydration processing using $CaCl_2$ or silica gel have a positive effect to

increase the contents of phenols, it resulted in the highest value 123.69 mg/100 g F.W. for the (D1) but decline slightly to 92.5 for the second. Furthermore, all the other treatments resulted decrease in their value compared with control, it ranged between 14.007 and 37.905 mg/100 g F.W. In general, the highest value of the endogenous total phenol in *R. polyantha* explants were found in dehydration under $CaCl_2$ conditions.

The results on **Table (13)** demonstrated that the total soluble indoles were determined on the pretreatment of cryopreservation on *A. salicina* explants, it resulted in a significant difference between the treatments. The highest value was assayed 598.02 mg/100 g F.W for the vitrification (V1) compared to 259.58 mg/100 g F.W for the control. This effect as trend the explant exposed to vitrification (V3) and pregrowth (PII). 319.45 and 360.046 mg/100 g F.W respectively. However, all the other treatments resulted decrease in the level of endogenous indoles. In this treatment for *Ceratonia siliquia*, only dehydration under $CaCl_2$ resulted the high value (514.59 mg/100 g F.W.) compared with 383.11 for the control. Meanwhile, the lowest value was 153.32 for vitrifiaction (V3) .All the other treatments resulted in slightly decrease as it ranged between 170.7 and 284.08. On *Rosa polyantha* the endogenous of total soluble indoles of the exposed the explants to drying condition under $CaCl_2$ resulted in highest value 819.455 mg/100 g F.W .This effect as trend to the explants treated with vitrification (V4), it was 517.03 mg/100 g F.W. In general, the highest contents of total soluble indoles in *R. polyantha* for all treatments compared with *C. siliquia* and *A. salicina*, but the highest level of total soluble indoles was observed on dehydration under $CaCl_2$ conditions

Table (13): Effect of some pretreatment of cryopreservation on some chemical compositions of *Acacia salicina in vitro* explants after soaking in liquid nitrogen

Treatments	Total Phenol	Total indols	Total carbohydrates	Chlorophyll - a	Chlorophyll- b	Total carotenoids	POD	PPO
Control	11.41	259.58	44.65	92.39	48.22	77.10	86.10	19.75
$CaCl_2$ dehydration	5.85	178.17	38.94	81.99	51.03	85.22	163.37	11.85
Silica gel dehydration	2.20	206.53	23.85	65.91	36.68	64.55	77.42	11.86
PVS1	4.50	598.02	21.06	139.67	48.09	119.06	177.43	12.35
PVS2	4.26	237.17	14.59	88.11	34.49	112.47	69.29	16.12
PVS3	2.78	319.45	17.13	160.57	67.99	143.73	15.61	9.67
PVS4	4.59	85.23	23.47	58.43	26.77	77.15	43.35	29.80
PI	10.35	213.69	38.12	66.94	28.33	60.59	77.21	11.88
P II	14.62	360.05	39.83	33.26	15.42	55.17	29.50	18.08
E / D I	4.61	148.45	14.97	51.55	18.20	49.26	178.92	5.75
E/DII	3.59	242.89	11.80	84.41	26.73	108.53	177.99	5.39
L.S.D. 5 %	0.56	25.36	0.25	1.25	0.62	4.21	22.36	0.95

Table (14): Effect of some pretreatment of cryopreservation on some chemical compositions of *Ceratoni siliqua in vitro* explants after soaking in liquid nitrogen

Treatments	Total Phenol	Total indols	Total carbohydrates	Chlorophy ll - a	Chlorophy ll- b	Total carotenoids	POD	PPO
Control	14.12	383.11	27.34	126.03	48.60	150.31	65.41	0.96
$CaCl_2$ dehydration	38.95	514.59	40.47	322.68	161.39	284.22	36.08	7.85
Silica gel dehydration	28.09	233.21	30.44	156.60	77.19	122.01	43.02	2.00
PVS1	20.25	203.33	28.54	142.87	122.11	172.52	26.58	5.05
PVS2	6.62	210.98	27.27	139.89	54.41	114.15	30.16	4.51
PVS3	6.58	153.32	24.74	66.52	43.67	62.19	40.07	4.94
PVS4	14.39	213.08	29.68	46.79	34.39	71.29	20.34	16.87
PI	7.85	284.09	30.70	76.52	35.51	106.50	160.57	3.30
P II	3.92	269.49	35.65	64.15	29.92	67.65	16.60	5.15
E/DI	5.86	170.70	34.95	71.82	43.40	72.25	14.85	5.16
E/DII	2.57	275.55	10.78	87.51	24.39	75.96	30.98	8.25
L.S.D at 5%	0.89	47.89	9.80	14.321	2.73	10.423	127.95	0.899

The results in **Table (14)** revealed that the total carbohydrates were determined in the cell extraction from *C. siliqua*, the highest value were recorded for the desiccated explants under $CaCl_2$, it was 40.46 mg/100 g F.W compared to 27.33mg/100 g F.W. for the control . All the other treatments induced an increase in their value except in vitrification V2 and V3 or encapsulation dehydration under silica gel, it ranged between 10.78mg/100 g F.W. and 27.27 mg/100 g F.W. On the other hand, in *Acacia salicina* the highest value were 44.65 for soaking the explants in liquid nitrogen directly. Both of dehydration under $CaCl_2$ or pregrowth on cumarin at 1.5 mg/l (PI) and pregrowth under DMSO and glycerol have a non-significantly differences in results 38.94, 38.11 and 39.83mg/100 g F.W. Moreover, in *R. polyantha* the amount of total carbohydrates were accumulated in the cell for the explant dehydrated under $CaCl_2$ or silica gel 58.6 and 58.03 mg/100 g F.W. Respectively, but the lowest value were 13.701 for encapsulation dehydration under $CaCl_2$ stress.

On oxidation processing under various pretreatment of cryopreservation for *Acacia salicina* explants this effect were tabulated in **Table (13)** It revealed that the activity of polyphenoloxidase enzyme which determined for the *in vitro* explants exposed to liquid nitrogen, The highest activity value were observed for the explants soaked in cryoprotactants solutions (PVS4) 29.8 Unit/ml.min compared to 19.75 Unit/ml.min for the control. Meanwhile, all the other pretreatment resulted in sharply decrease in their activity of polyphenoloxidase. The explants treated with encapsulation dehydration under silica gel were recorded the lowest value (5.39 Unit/ml.min). On the other hand, the activity of PPO for the *C. siliquia* exposed to various pretreatment gave the highest activity of PPO for the explants soaked in cryoprotactant solution (PVS 4) it was recorded 16.87 Unit/ml.min. The lowest value were recorded for the control 0.96 Unit/ml.min. On the other hand, all the other pretreatments resulted in increase to the PPO activation compared with the lowest value (0.96 Unit/ml.min) for the control. Moreover, Data presented in **Table (15)** showed that the

highest units of activation of PPO for *Rosa polyantha* explants *in vitro* plunged directly in liquid nitrogen (control) without any pretreatments, it was 70.7 Unit/ml.min. Meanwhile, the lowest value were recorded 0.75 Unit/ml.min for the explants exposed to encapsulation dehydration under CaCl2 it was 0.37 Unit/ml.min

Data presented in **Table (13)** showed that the activation of the Peroxidase (POD) in *in vitro Acacia salicina* explants after soaking in liquid nitrogen. The highest oxidation units of POD were observed under encapsulation dehydration under $CaCl_2$ it was recorded 178.92 Unit/ml.min. Moreover, the lowest value observed were 15.61 Unit/ml.min for the explant soaked in cryoprotactant solution (PVS 3) both of drying under $CaCl_2$, encapsulation dehydration under silica gel and soaking in cryoprotactant (PVS 1) gave an increase in the POD activation compared to 86.1 Unit/ml.min On the other hand, Data presented in **Table (14)** revealed that the highest number of POD oxidation recorded for the explants treated with pregrowth (I) it was 160.57 Unit/ml.min. Meanwhile, the lowest value were resulted 14.85 Unit/ml.min for the explants exposed to encapsulation dehydration under $CaCl_2$. However, all the other treatments resulted in decrease in their value compared to 65.41 Unit/ml.min for the control Data presented in **Table (15)** showed that in *Rosa polyantha* the enzyme activity of POD were determined for all the pretreatments, soaked in cryoprotactant solution (PVS4) resulted in high value 35.67 Unit/ml.min. Moreover, the lowest value was observed in the explants exposed to silica gel, it was (4.16).

Table (15): Effect of some pretreatment of cryopreservation on some chemical compositions of *Ceratonia siliqua in vitro* explants after soaking in liquid nitrogen

Treatments	Total Phenol	Total indols	Total carbohydrates	Chlorophyll - a	Chlorophyll- b	Total carotenoids	POD	PPO
Control	53.97	499.65	39.26	309.64	157.19	250.64	5.45	70.70
$CaCl_2$ dehydration	123.69	819.46	58.60	363.20	158.27	350.08	5.93	0.77
Silica gel dehydration	92.51	385.66	58.03	243.55	102.19	242.69	4.16	1.36
PVS1	31.71	237.63	26.96	143.16	68.55	136.52	13.03	0.46
PVS2	14.01	246.35	22.71	144.73	72.33	139.27	9.13	2.24
PVS3	35.35	360.35	32.60	227.35	80.60	232.25	7.31	2.66
PVS4	37.91	517.03	24.86	220.47	127.47	341.77	35.67	2.68
PI	16.91	457.27	23.34	347.34	112.16	292.93	4.84	1.11
P II	28.80	264.46	38.75	138.82	56.44	138.21	13.74	0.71
E / D I	16.22	336.53	13.70	112.02	47.17	126.00	19.97	0.37
E / D II	17.32	340.19	26.77	94.02	38.68	85.76	33.63	0.75
L.S.D 5 %	0.5879	23.254	2.54	35.24	12.36	40.3	8.245	0.9547