RESULTS

The main objective of the present studies is to produce a virus-free potato plants by using tissue culture technique alone or combined with other treatments. These studies were started by detection of potato virus X (PVX) in diseased plants of different potato varieties grown from local seed tubers under natural conditions (Fig. 2) of Behera and Menoufia Governorates. A lot of leaf samples were taken from an individual plants of such potato varieties showing different degrees of mosaic symptoms. Sap obtained from such leaves and also from leaf samples showing no symptoms was used for PVX-detection by rub transmission on PVX-indicator plants.

Mechanical inoculation tests showed that sap expressed from potato leaves with mild, clear mosaic or even healthy looking taken from all the afore mentioned potato varieties gave brownish local lesions on leaves of Gomphrina globosa plants (Fig. 3). The lesions began to appear on the 6th day from inoculation with sap from leaves with clear mosaic symptoms collected from Alpha, Diamont and Spunta potato varieties. Development of local lesions was delayed until the 9th when sap was obtained from leaves of Cara, Desiree, Foundal and Jaerla potato varieties either bearing mild, clear mosaic or even healthy-looking appearance. On the other hand, sap taken from all collected leaf samples induced systemically mild mosaic symptoms when mechanically inoculated onto lower leaves of Datura stramonium and Nicotiana tabacum var. White Burley (Fig. 4). Mosaic symptoms developed 15 and 20 days from inoculation of the two indicator hosts, respectively.

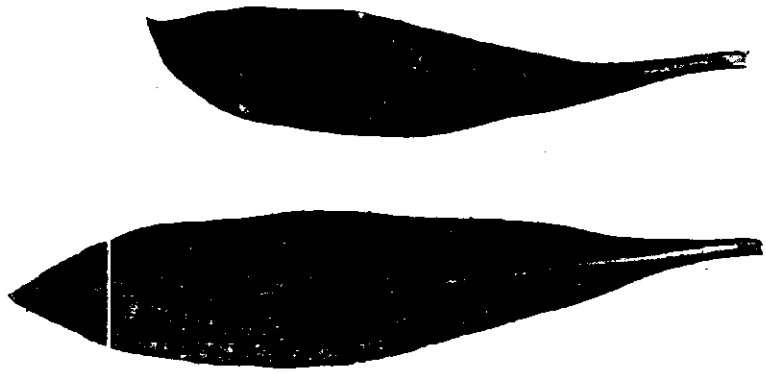


Fig. (3): Local lesions formed on leaves of G. globosa.

Fig. (2): Severe mosaic symptom on potato plants (natural infection).

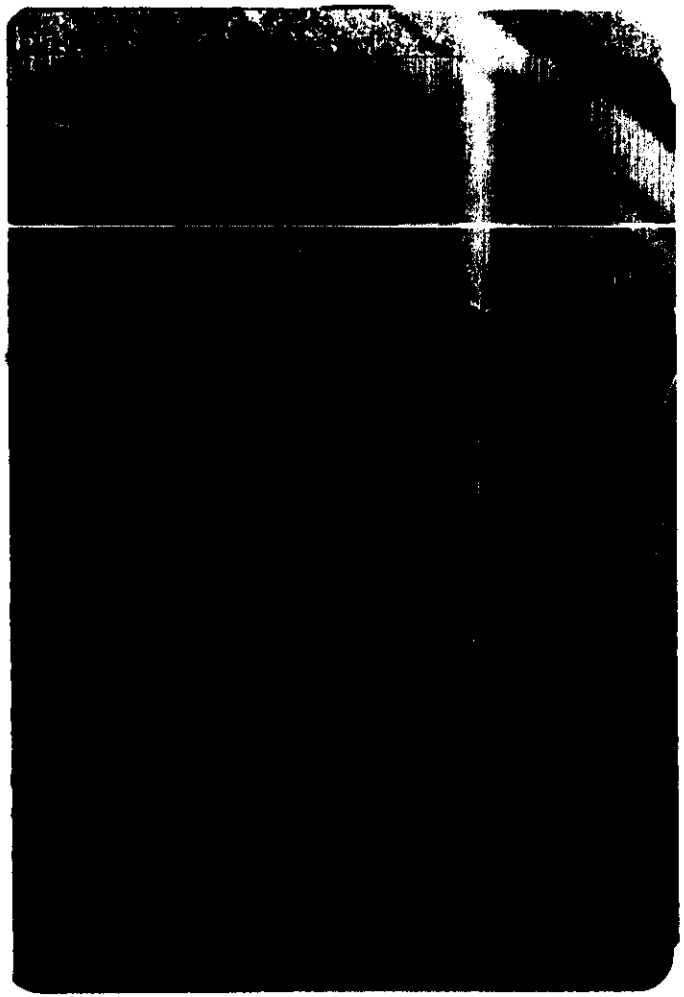


Fig. (4): Mild mosaic on leaves of *Nicotiana tabacum* var White Burley.

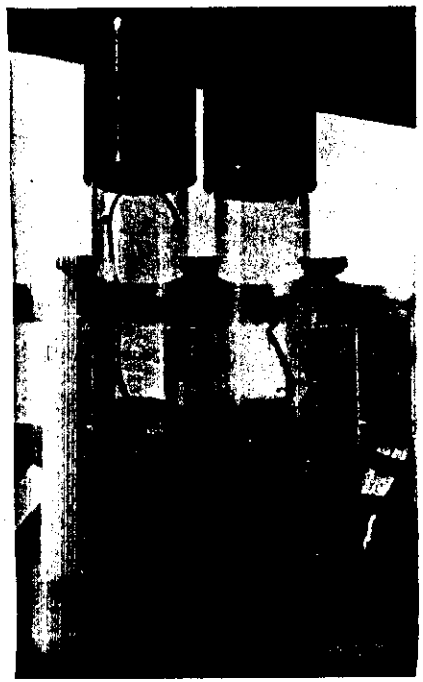


Fig. (5): Growth and development of meristem tips, 0.2 mm (right) and 0.4 mm long (left) of Alpha var.

Back inoculation from the latter two infected systemic hosts to G. globosa resulted also in local lesions formation.

Generaly, as local lesions and systemic mosaic symptoms appeared on specific PVX-indicator plants, we can say that potato virus X could be detected in all leaf samples taken from labeled plants of different examined potato varieties grown under field conditions. On other means, all selected potato plants proved to be 100% infected with PVX. The different patterns of disease symptoms might be due to more than one of PVX-strain.

Effect of size of explant:

Two different lengths of meristem tip i.e. 0.2 and 0.4 mm long were excised, as mentioned in Materials and Methods, from potato plants of different varieties which completely infected with PVX. The excised meristem tips were cultured on solid nutrient medium containing basic MS-salts and vitamins (Murashige and Skoog, 1962) and incubated for 5 weeks as mentioned before. The different effects of size of explant could be summarized as follows:

a. Effect on survivals and relative growth:

The number of survived meristem tip cultures and their relative growth rates as affected by size of excised meristem tips for each tested potato varieties are summarized in Table(2).

Regardless potato varieties the larger excised meristem tips i.e. 0.4 mm gives the higher percentage of survivals (49.4%) when compared with those survived from the smaller

Table (2): Effect of size of explant (mm) on survivals and rate of growth of cultured meristem tips taken from different potato varieties.

Variety	Size of explant (mm) 0.2 S/T (a)	%	0.4 S/T	%	Relative growth scores for tip with length of: 0.2 mm	0.4 mm	L.S.D. at 0.05 (b)
Alpha	20/48	41.7	21/48	43.8	2.8	4.5	0.72
Cara	18/48	37.5	21/48	43.8	3.4	3.9	n.s.
Diamont	24/48	50.0	28/48	58.3	3.3	3.9	n.s.
Desiree	15/48	31.3	23/48	47.9	4.4	4.7	n.s.
Foundal	14/48	29.2	19/48	39.6	2.7	4.3	0.61
Jaerla	16/48	33.3	24/48	50.0	4.0	4.8	0.53
Spunta	21/48	43.8	30/48	62.5	3.0	4.0	0.51
Total	128/336	-	166/336	-	-	-	-
Mean	-	38.1	-	49.4	3.4	4.3	-

(a) S/T is the survivals/total cultured tips.

(b) A one way AOV analysis was performed on each variety and L.S.D was applied to compare between rate of growth of the two meristem tip lengths when justified by a significant F value in the AOV.

meristem tips, 0.2 mm long (38.1%). The percentages of survivors clearly depended on both potato variety and size of meristem-tip. When meristem tips 0.2 mm long were cultured, the highest survival percentage (50%) was associated with Diamont variety, In contrast, the best survivals (62.5%) were in meristem tips 0.4 mm long excised from Spunta variety. However, potato variety Foundal gives the lowest survivals i.e 29.2% and 39.6% in case of 0.2 and 0.4 mm long meristem, respectively.

Regardless the size of meristem tips, potato varieties could be arranged descendingly at the base of survived cultures as follows, Diamont, Spunta, Alpha, Jaerla, Cara, Desiree and Foundal.

Comparisons of growth ratings for the two lengths of meristem tips indicated that development and relative growth was significantly delayed in most potato varieties when small meristem tips were used. The highest reduction in growth was observed with the small meristem-tips 0.2 mm long of potato varieties Alpha (Fig. 5) and Foundal (Table 2). In case of Cara and Diamont, the rate of growth was clearly delayed especially with the large 0.4 mm long meristem tips when compared with the other varieties.

b. Effect on development of plantlets:

Data in Table (3) clearly show that the number of developed plantlets and their vegetative characters were

Table (3): Effect of size of explant (mm) taken from different potato varieties on the number of developed plantlets and its vegetative characters.

Size of explant (mm)	Variety	Plantlets/total cultured tips		Vegetative characters of plantlets developed (average)		
		No. D/T*	%	Stem length (mm)	No. of leaves (mean)	No. of roots (mean)
0.2	Alpha	7/48	14.6	26	4.4	3.4
	Cara	3/48	6.3	16	4.0	2.7
	Diamont	7/48	14.6	32	5.1	4.6
	Desiree	14/48	29.2	35	5.3	3.6
	Foundal	8/48	16.7	12	3.6	2.6
	Jaerla	10/48	20.8	22	5.8	2.1
	Spunta	8/48	16.7	21	4.5	3.0
Total		57/336	-	-	-	-
Mean		-	17.0	23.4	4.7	3.1
0.4	Alpha	16/48	33.3	48	5.9	3.6
	Cara	14/48	29.2	25	5.6	3.0
	Diamont	18/48	37.5	62	8.9	6.0
	Desiree	21/48	43.8	48	7.6	3.7
	Foundal	17/48	35.4	53	6.4	4.0
	Jaerla	23/48	47.9	34	6.3	2.8
	Spunta	23/48	47.9	21	5.2	3.6
Total		132/336	-	-	-	-
Mean		-	39.3	41.6	6.6	3.8

* Number of developed plantlets/total cultured tips.

greatly affected with the length of the cultured meristem tips. Generally, all potato varieties exhibited higher reduction in the number of developed plantlets, means of stem length, leaves and root number per plantlets when the small meristem tips were cultured than the large one. These vegetative measurements as well as number of developed plantlets were differed greatly among potato varieties as well as between the two sizes of meristem tips. But there is no clear relation between such measurements and number of developed plantlets. Regardless varieties, 57 and 132 plantlets were obtained from tips with 0.2 and 0.4 mm long,respectively. The variety Cara showed the lowest number of plantlets developed either from the small and large meristem i.e 3 (6.3%) and 14 (29.2%) plantlets respectively. The highest figure was in Desiree developed from the small meristem tips i.e 14 plantlets (29.2%) and Jaerla and Spunta developed from the large meristem i.e 23 plantlets in both (47.9%) were developed.

c. Effect on inviability and contamination with microorganisms:

Effect of size of meristem tips on culture inviability and occurrence of contamination with microorganisms was shown in Table (4). Data clearly indicated that using meristem tips either 0.2 mm or 0.4 mm long resulted in great increase in cultures lost due physiological death or bacterial and fungal contamination, but losses due to the first reason were higher i.e. (154/336) and (123/336) than those arised from the later one i.e. (54/336) and (47.336) in case of small and large explant, respectively. The growth of the most lost cultures

Table (4): Effect of size of explant (mm) taken from different potato varieties on the number of cultures lost due to inviability and contamination.

Variety	Size of explant (mm)							
	0.2 mm				0.4 mm			
	No. losses due to				No. Losses due to			
	inviability*	Contamination	Total losses	%	inviability*	contamination	Total losses	%
Alpha	**26/48**	2/48	28/48**	58.3	23/48	4/48	27/48**	56.3
Cara	**18/48**	12/48	30.48	62.5	18/48	9/48	27/48	56.3
Diamont	9/48	15/48	24/48	50.0	9/48	11/48	20/48	41.7
Desiree	25/48	8/48	33/48	68.8	18/48	7/48	25/48	52.1
Foundal	28/48	6/48	34/48	70.8	23/48	6/48	29/48	60.4
Jaerla	30/48	2/48	32/48	66.7	24/48	0/48	24/48	50.0
Spunta	18/48	9/48	27/48	56.3	8/48	10/48	18/48	37.5
Total	154/336	54/336	208/336	-	123/336	47/336	170/336	-
%	45.8	16.1	61.9	-	36.6	14.0	50.6	-

* All counted cultures were turned brownish and dead without any fungal or bacterial contamination.

** Numerator, is the number of cultures lost, Denominator is the total tips culture.

Infected Healthy

Fig. (6): PVX-infected and virus-free potato plants after 3 weeks from transplanting.

was early stopped then turned brown and quickly diet. In case of contamination with fungi and bacteria, the cultured meristem tips may grow and developed into plantlets, thereby, they are finally excluded. However, potato varieties differed in the rate of inviability. Potato varieties Diamont and Jaerla showing the lowest and highest losses due to inviability of meristem tips of both sizes, respectively. The reverse pattern of losses occurred due to contamination. On the other hand, Jaerla and Alpha varieties resulted in the lowest numbers of cultures lost due to contamination for both sizes of meristem tips.

d. Effect on production of virus-free plantlets:

Plantlets which developed from culturing meristem tips with lengths of 0.2 and 0.4 mm, were indexed for the presence of PVX three times using the rub transmission test onto leaves of Gomphrina globosa plants. The first indexing was carried out using in vitro plantlet's shoot portions that remained after subculturing of plantlets. The 2nd and 3rd indexings were after 5 and 10 weeks from transplanting of plantlets regenerated from subcultures (Fig. 6) (Materials and Methods).

Data about the 1st and last indexings were tabulated in Table (5). Data clearly indicated that out of 166 and 128 survivals 132 and 57 were successively developed into plantlets when the large and small meristem tips were used, respectively. However, the numbers of plantlets indexed free from PVX were approximately unchanged in both lengths of

Table (5): Effect of size of meristem tips excised from different potato varieties infected with PVX on elimination of this virus and number of PVX-free plantlets obtained.

Variety	0.2 mm meristem tip					0.4 mm long meristem tip				
	D/S*	%	Virus-free test 1st[1]	Virus-free test 3rd[2]	Virus-free % after 3rd test	D/S	%	Virus-free test 1st	Virus-free test 3rd	Virus-free % after 3rd test
Alpha	7/20	35.0	4/7	2/7	28.6	16/21	76.2	5/16	2/16	12.5
Cara	3/18	16.7	3/3	2/3	66.7	14/21	66.7	4/14	4/14	28.6
Diamont	7/24	25.9	4/7	3/7	42.9	18/28	64.3	5/18	4/18	22.2
Desiree	14/15	93/3	9/14	8/14	57.1	21/23	91.3	6/21	6/21	28.6
Foundal	8/14	57.1	6/8	5/8	62.5	17/19	89.5	5/17	4/17	28.5
Jaerla	10/16	62.5	7/10	5/10	50.0	23/24	95.8	5/23	5/23	21.7
Spunta	8/21	38.1	2/8	2/8	25.0	23/30	76.7	4/23	3/23	13.0
Total	59/128	-	35/57	27/57	-	132/166	-	34/132	28/132	-
%	44.5	-	61.4	47.4	-	79.5	-	25.8	21.2	-

* D/S = Number of developed plantlets/total survivals.
1=1st = Number of initially PVX-free plantlets/No. of developed plantlets.
2=3rd = Number of plantlets undexed PVX free after 10 weeks from transplanting in pots/No. of developed plantlets.

meristem tips, these were 27 out of 57 and 28 out of 132 for the small and large meristem tips, respectively. But it could be observed clearly that the percentages of such PVX free plantlets were higher in the small than the large meristem tips, they were 47.4% (27/57) and 21.2% (28/132), respectively.

As regards potato varieties, data in Table (5) showed that the number of plantlets developed ranged between 3-23 for the different varieties. Potato variety Cara resulted in the lowest numbers of developing plantlets for both lengths of meristem tips as they were 3 and 14 plantlets for the small and large ones, respectively, however the same variety gave the highest percentages of PVX-free plants i.e. 66.7% and 28.6% for the small and large meristem, respectively. On the other hand, variety Spunta resulted in the highest developing plantlets, however it gave the lowest percentages of PVX-free plantlets i.e 25.0% and 13.0% for the small and large meristem tips, respectively. In general, PVX could be eliminated from potato varieties Cara, Foundal, Desiree and Jaerla at relatively high rate of 66.7%, 62.5%, 57.1% and 50.0% respectively when meristem tips 0.2 mm long were used. With meristem tips 0.4 mm long, the corresponding figures for the same above varieties were decreased to 28.6%, 23.5%, 28.6% and 21.7%, respectively.

Data showed also that the most plantlets infected with PVX could be detected through the first indexing test. In some

potato varieties few infected plantlets could be escaped from the first indexing as the virus detecting was delayed few weeks later. Generally, all infected plantlets were successfully detected on the second indexing test. All PVX free ones gave negative results on the third indexing test, which carried out 10 weeks from transplanting in pots.

Effect of heat treatment:

Preliminary study was carried out to investigate heat therapy on eradication of potato virus X (PVX) from meristem tips with 0.4 mm long, isolated from potato plants infected with this virus. The excised meristem tips were cultured on solid medium containing MS-salts and vitamins of Murachiage and Skoog's medium, supplemented with 0.1 mg/l GA_3 and 2.0 mg/l calcium pantothenate then incubated at temperature of 37 ± 1°C. After more than 5 weeks from incubation, no plantlets rather that any growth expressions were observable. All cultured meristem tips turned brownish and died after 3-5 days from incubation at the temperature used.

This result concluded that combination between heat-therapy and meristem tip culture at both temperature and length of meristem tip used herein was practically impossible procedure.

In a separate experiment, effect of heat therapy on elimination of PVX was carried out in different procedure, in which meristem tips were replaced by nodal stem segments.

The later were taken from in vitro plantlets 5 weeks-old and cultured or media like that above mentioned (see Materials and Methods). After pre-heat conditioning period, the cultured nodal segments were left for 2, 4 and 6 weeks at 37±1°C in an incubator (Percival-MFG Co. Boone. Iowa Model 1-35 LLVL) under 16 h and 8 h of light and darkness respectively. It was hoped that this procedure might increase the rate of growth of nodal segments as well as the apical portion of meristematic tissues that become free from PVX, so as to enable us to excise a longer pieces of meristems. Meristem tips of 1.0 mm long were used, such tips were excised from shoots which developed during the prolonged heat therapy or during the heat-conditioning as for control treatment. Results obtained and presented in Figure (8) and Tables 6, 7, 8, 9 and 10 could be summarized as follows:

a. Effect of heat treatment on relative growth:

Data presented in Table (6) and Fig. (7) illustrated that the average of relative growth of potato nodal segments was clearly decreased with increasing period of heat treatment from 2 to 6 weeks when compared with that of control treatment. The different potato varieties were differed in their values of relative growth and sensitivity to heat therapy. In case of potato varieties Desiree, Foundal and Jaerla, could be considered the most susceptible to heat therapy as no growth had occurred at all when their nodal segments were incubated at 37±1°C for 6 weeks. The variety Foundal has the lowest values of relative growth when compared with the other

Table (6): Effect of prolongation of heat therapy (37°C±1) on the relative growth of nodal stem segments of different potato varieties.

Varieties	Relative growth scores (1-5) at periods of heat therapy "in weeks" 0	2	4	6	Mean	L.S.D at 0.5
Alpha	4.61	4.40	4,00	3.75	4.19	0.31
Cara	4.79	4.76	4.62	4.44	4.65	N.S.
Diamont	4.50	4.38	4.20	4.00	4.27	"
Desiree	4.91	4.55	4.18	-*	4.55	"
Foundal	3.91	3.73	3.56	-	3.73	"
Jaerla	4.80	4.50	4.37	-	4.56	"
Spunta	4.70	4.71	4.60	3.64	4.41	0.28
Mean	4.60	4.43	4.22	3.96	-	-

(-) All shoots that developed during heat treatment were dead. These varieties seems to be intolerant to prolongation of heat upto 6 weeks.

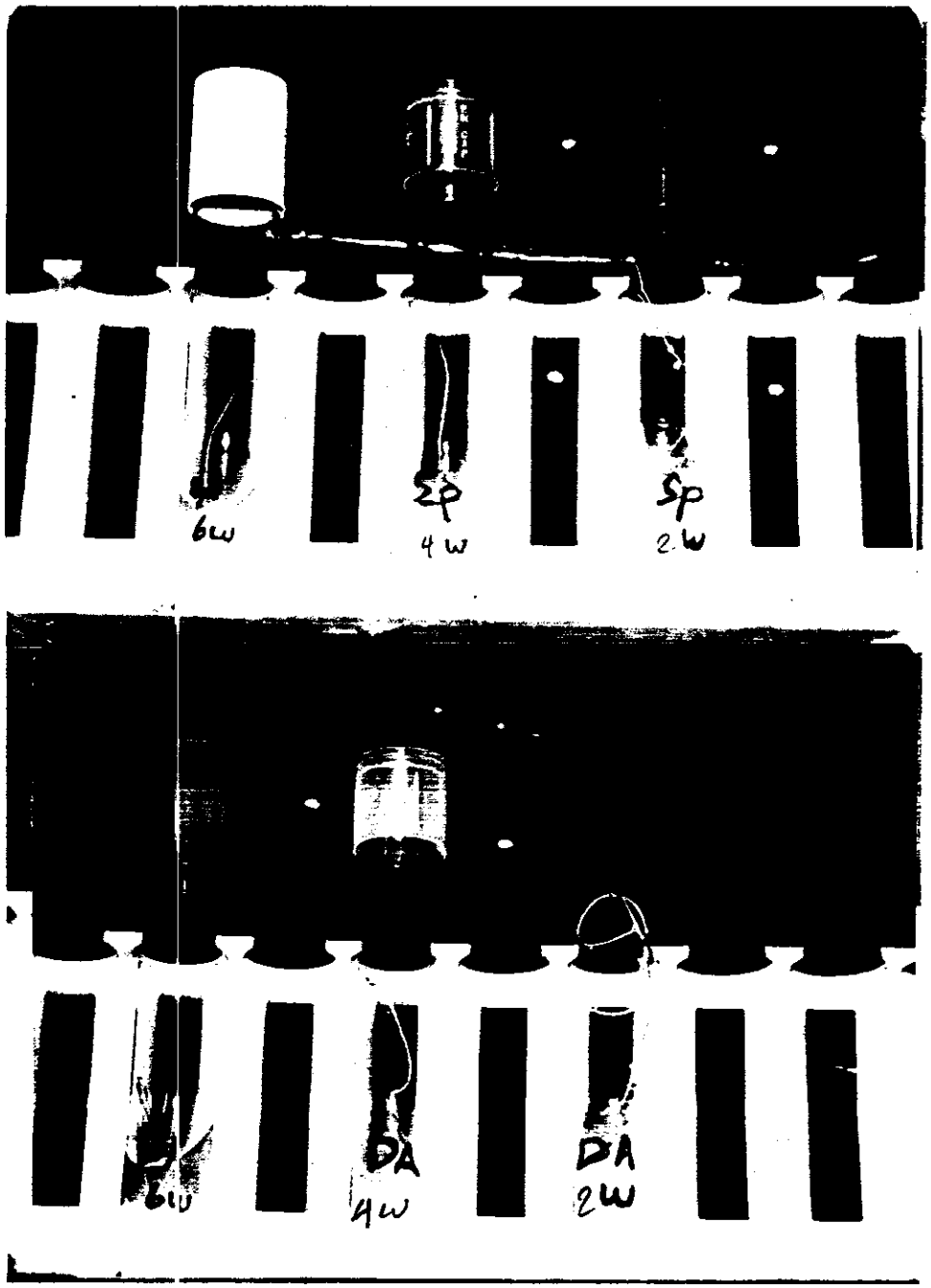


Fig. (7): Heat therapy treatment.
(Continued).

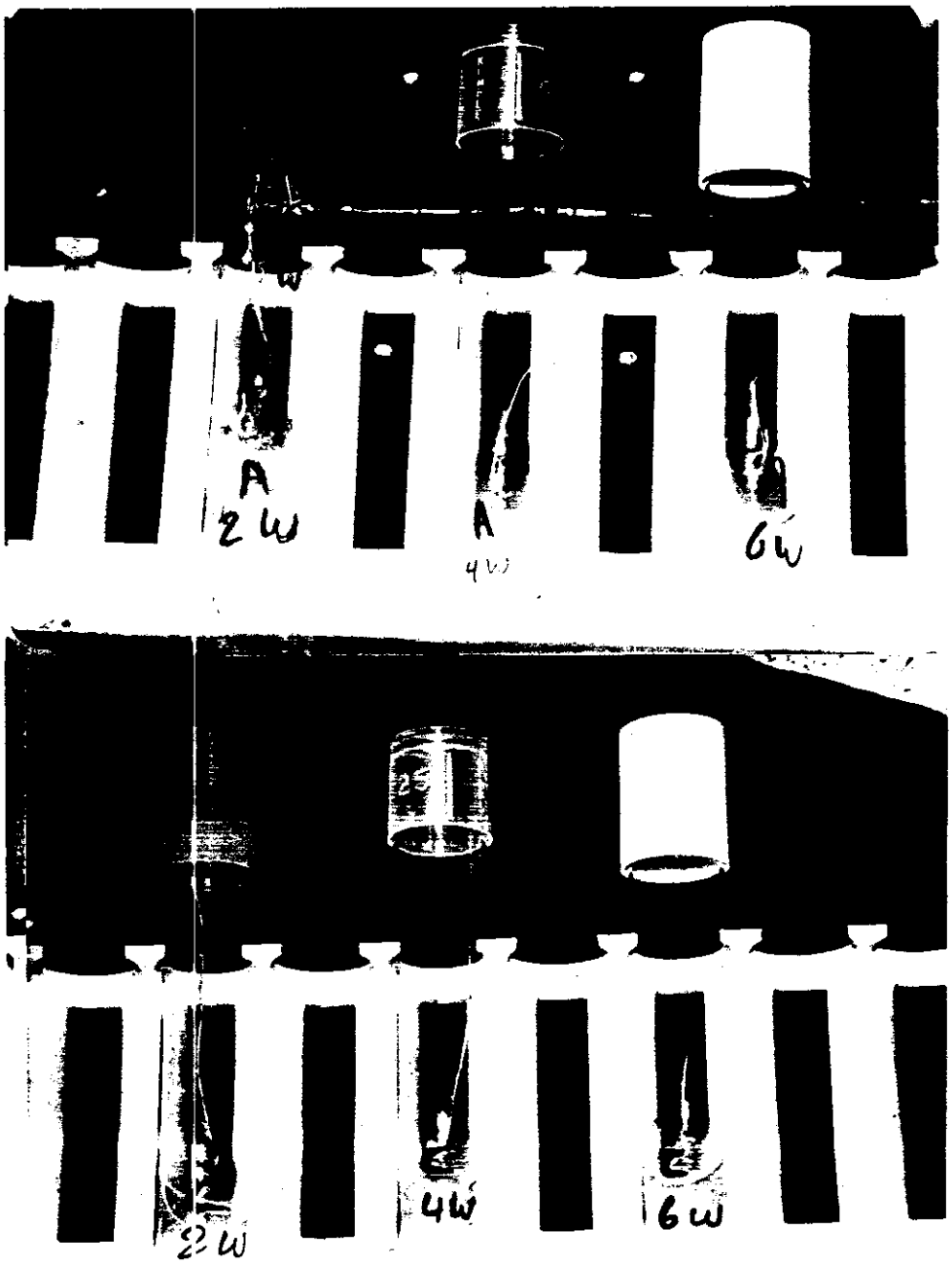


Fig. (7): Effect of prolongation of heattherapy on the rate of growth of nodal segments.

different tested varieties. On the other hand, relative growth of varieties Alpha and Spunta was significantly decreased with increasing heat-therapy up to 4 and 6 weeks respectively. Fig. (7) shows also, the reduction in growth rate of nodal segments of different potato varieties as a result of heat therapy prolongation.

b. Effect on culture inviability:

After plonged periods of heat therapy, meristem tips of 1.0 mm long were excised from the new shoot(s) developed on nodal segments during heat treatment, cultured and placed in an incubator to grow into plantlets. To find any relation(s) between duration of heat therapy and cultures viability, the number and percentages of inviable cultures were counted after 5 weeks from excision. The data tabulated in Table (7) show that many cultured meristem tips failed to grow. Out of total 1200 cultures for all varieties 271 were inviable (22.6%). The percentages of inviability increased from 18.5% to (32.3)by increasing heat therapy from 2 to 6 weeks respectively, these were more than those of control treatment (15.5%). The lost in cultures due to inviability seems to be also, depended on potato varieties. In this respect, they could be arranged descendingly as Spunta, Cara, Diamont, Alpha, Foundal Jaerla and Desiree. For the last 3 varieties all cultures used after 6 weeks heat therapy were lost due to inviability. On the other hand, the varieties Spunta and Cara seems to be more resistant against heat therapy prolongation. However, inviability of Alpha variety exhibited

Table (7): Effect of prolongation of heat therapy on the inviability of cultured shoot tips 1.0 mm long, excised from shoots developed during heat treatment.

Varieties	No. of cultured tips for each period	No. of inviable cultures after different periods of heat therapy "in weeks".				Total	%
		0	2	4	6		
Alpha	48	9	3	15	12	39/192**	20.3
Cara	48	7	9	9	19	44/192	22.9
Diamont	48	6	11	12	11	40/192	20.8
Desiree	48	8	9	12	-*	29/144	20.1
Foundal	48	6	12	21	-*	39/144	27.1
Jaerla	48	7	11	14	-*	32/144	22.2
Spunta	48	9	7	12	20	48/192	25.0
Total	336	52	62	95	62	271/1200	-
%		15.2	18.5	28.3	32.3		

* All excised shoot tips for such three varieties were unable to differentiated even up to more than 5 weeks after excissing, so it considered not present.

** Number of inviables/number of total tips cultured.

different reaction with different periods of heat treatment, as it was clearly decreased then sharply increased with 2 and 4 weeks heat therapy, respectively, compared with control treatment.

C. Effect on survivals and plantlet development:

Data in Table (8) clearly show that culture survivals were highly affected by heat therapy and by potato varieties. Out of 1344 cultures for different varieties 801 only have survived (59.6%). The short heat therapy i.e 2 weeks has little effect on survivals when compared with the control treatment. The survivors were 241 and 248 out of 336 cultures for these two treatments, respectively. However, increasing heat therapy up to 4 and 6 weeks decreased survivors to 201/336 and 111/336, respectively. With respect to potato varieties, there was no observable differences between them either in number or percentages of survivals, however, they reacted differently with prolonged periods of heat treatment. Generally, survivals of most varieties were decreased with heat therapy of 2 weeks and more except Alpha and Spunta varieties as their survivals were increased with 2 weeks of heat therapy if compared with control treatment. On the other hand Foundal, Desiree and Jaerla varieties resulted in the lowest survivals because inviability of their cultures of 6 weeks heat therapy.

Regarding developed plantlets, data also show that the number of plantlets developed after heat therapy treatments has sharply decreased compared with that of

Table (8): Effect of heat treatments on the number of developed plantlets in proportion to survival cultures originated from shoot tips 1.0 mm long, excised after different periods from heat treatment.

Varieties	No. of cultured tips for each period	No. of plantlets/survivals after different periods of heat therapy "in weeks"				Total	%
		0	2	4	6		
Alpha	48	29/33	27/40	17/28	10/27	83/128	64.8
Cara	48	31/36	28/35	24/32	21/26	104/129	80.6
Diamont	48	20/37	18/33	18/27	22/32	78/129	60.5
Desiree	48*	33/37	24/31	20/30	0/0	77/98	78.6
Foundal	48*	20/37	12/31	11/23	0/0	43/91	47.3
Jaerla	48*	32/36	22/33	22/30	0/0	78/99	78.8
Spunta	48	26/32	15/38	23/31	20/26	84/127	66.1
Total	336	191/248	146/241	135/201	73/111	547/801	-
%	100	77.0	60.6	67.2	65.8	68.3	

* At treatment of 6 weeks heat therapy, all processes were not carried out for such three varieties.

control. After 0, 2, 4 and 6 weeks heat treatment, plantlets developed/survivors were 191/248, 146/241, 135/201, and 73/111, respectively. With contrast to survivals, the number and percentages of developed plantlets clearly varied between potato varieties. Among different varieties, Cara one gave the highest figures, in it, out of 129 survivals 104 plantlets (80.6%) were obtained. The reverse was recorded in Foundal variety as 43 plantlets out of 91 survivals (47.3%) were developed.

d. Effect on elimination of PVX:

Results tabulated in Table (9) show that out of 545 developed plantlets 241 were PVX-free, of which 34.1% (186) were from different treatments of heat therapy compared with only 10.1% (55) resulted from control treatment. The average percentages of PVX-free plantlets were increased successfuly with increasing periods of heat treatment, these were 43.2% (63/146), 56.3% (76/135), and 64.4 (47/73) for periods of 2, 4 and 6 weeks heat therapy, respectively.

With regarding potato varieties, Foundal and Alpha gave the highest and lowest percentages of PVX-free plantlets, these were 74.4% and 25.3% for both varieties respectively. Although Cara variety gives only 44.2% healthy plantlets, but the corresponding number was the highest i.e 46 out of 104 plantlets were free from the virus. On the other hand, it could be noted that as the periods of heat therapy were prolonges, percentages of plantlets free from the concerned

Table (9): Effect of heat treatment for different periods "in weeks" on the number of PVX-free plantlets (a) out of all developed (b) in different potato varieties.

Varieties	Different periods of heat treatment at 37°C ± 1								Total a/b
	0 week		2 weeks		4 weeks		6 weeks		
	a/b	%	a/b	%	a/b	%	a/b	%	
Alpha	3/29	10.3	6/27	22.8	6/17	35.3	6/10	60.0	21/83
Cara	9/31	29.0	11/28	39.3	11/24	45.8	15/21	71.4	46/104
Diamont	7/20	35.0	9/18	50.0	10/18	55.6	14/22	63.6	40/78
Desiree	10/33	30.3	11/24	40.7	14/20	70.0	-*	-	35/77
Foundal	14/20	70.0	9/12	75.0	9/11	81.8	-*	-	32/43
Jaerla	8/32	25.0	10/22	45.5	15/22	68.2	-*	-	33/76
Spunta	4/26	15.4	7/15	46.7	11/23	47.8	12/20	60.0	34/84
Total	55/191	-	63/146	-	76/135	-	47/73	-	241/545
%	28.8	-	43.2	-	56.3	-	64.4	-	

* No plantlets at all were developed.

virus to be increased, but the numbers of such healthy plantlets were not reacted in the same manner. In other means, the number of healthy plantlets, in some instances were unchanged or not improved in some tested varieties. For example, in Alpha variety, 6 PVX-free plantlets were obtained in each of the three periods of heat treatment, however, this was twice that of control treatment. In the Foundal variety, the numbers of total as well as PVX-free plantlets were greatly decreased with heat therapy, in this variety out of 22 plantlets resulted in all treatments of heat therapy only 18 were PVX-free, while 14 out of 20 were healthy in the control treatment alone. In this respect, Foundal in addition to Desiree and Jaerla varieties seemed to be the most sensitive and indurable to heat therapy if compared with other tested varieties.

In general, tested potato varieties could be arranged descendingly according to success in eradication of PVX by using the previously described technique for heat therapy in comparison with their controls as follows: Spunta, Jaerla, Desiree, Alpha, Diamont, Cara and Foundal.

From explanation of our results, it could be concluded that:

1. Heat treatment at 37°C $\pm$ 1 was harmful to survivals when directly applied with meristem tip cultures. All cultured meristem tips quickly die when exposed to such temperature.

2. In vitro plantlet-nodal segment cultures combined with heat therapy, in most varieties resulted in highest number and percentages of PVX-free plantlets, with an exception of Foundal variety, in which exposing nodal segment cultures of 2 weeks old to heat-conditioning period of additional 2 weeks was highly beneficial in increasing number of virus-free plantlets of variety.

Chemotherapy: (Thiouracil treatment)

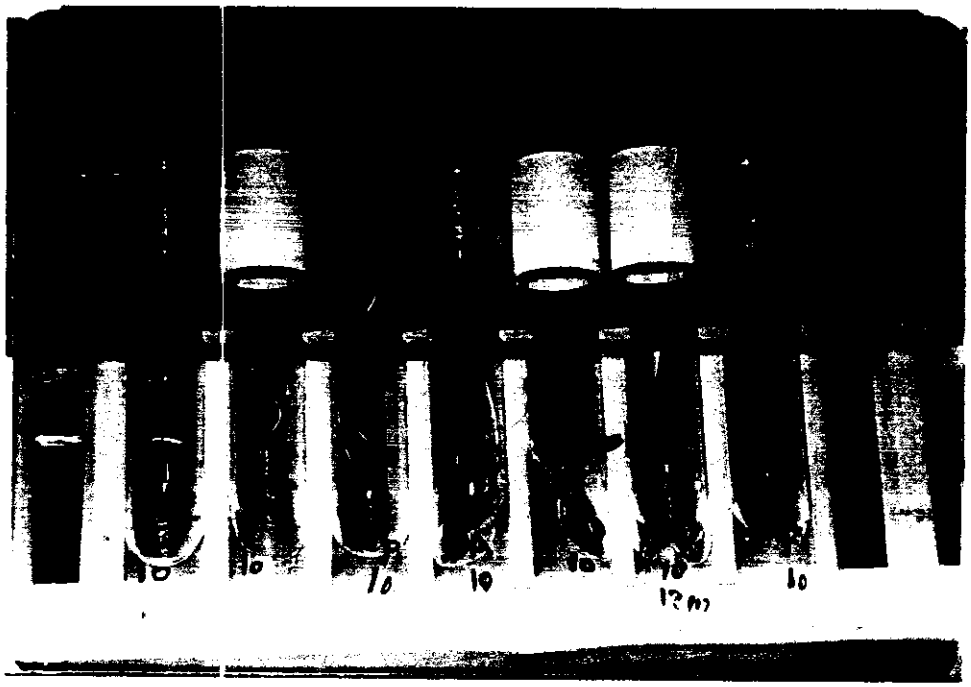
a. On relative growth:

In these studies, nodal stem segments taken from an in vitro plantlets of different potato varieties were cultured onto nutrient MS medium, into which, the thiouracil was incorporated at rates of 10, 20, 30, 50, 100 and 150 ppm. After incubation period of 5 weeks, individual nodal segment cultures were rated on modified relative growth scale as described early.

At first, all nodal segment cultures treated with either concentrations of 50, 100 or 150 ppm of thiouracil failed to survived and unable to develop into growing plantlets, thus, their corresponding zero values were canceled. The averages of relative growth for different potato varieties as affected by thiouracil at 10, 20 and 30 ppm are presented in Table (10) and Fig. (8). Regardless potato varieties, data in Table (10) indicated that thiouracil treatment clearly reduced growth even at the lowest concentration when compared with the non-treated control. Reduction in relative growth was increased proportionally with increasing concentrations of thiouracil. As for

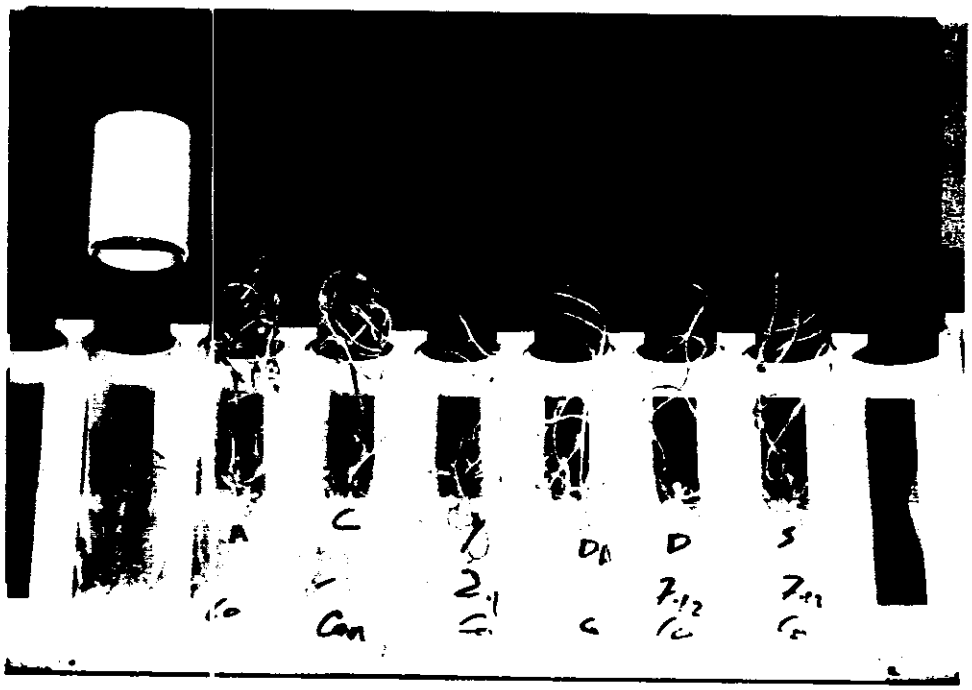
Table (10): Effect of different concentrations of thiouracil (in ppm) on average relative growth of nodal segment cultures of different potato varieties after 5 weeks from culture.

Varieties	Scores of relative growth at different concentrations(in ppm) of thiouracil				Mean	L.S.D at 0.05
	C	10	20	30		
Alpha	4.25	2.88	2.70	2.93	3.19	0.63
Cara	4.41	3.94	3.50	3.00	3.71	0.50
Diamont	4.60	3.93	3.20	2.63	3.59	0.67
Desiree	4.65	3.94	3.25	2.60	3.61	0.62
Foundal	4.25	3.50	2.94	2.44	3.28	0.58
Jaerla	4.33	4.20	3.27	3.13	3.73	0.53
Spunta	4.47	3.47	3.24	3.00	3.55	0.55
Mean	4.42	3.69	3.16	2.82	-	-



Thiouracil at 10 ppm

C Y SP DA D A F

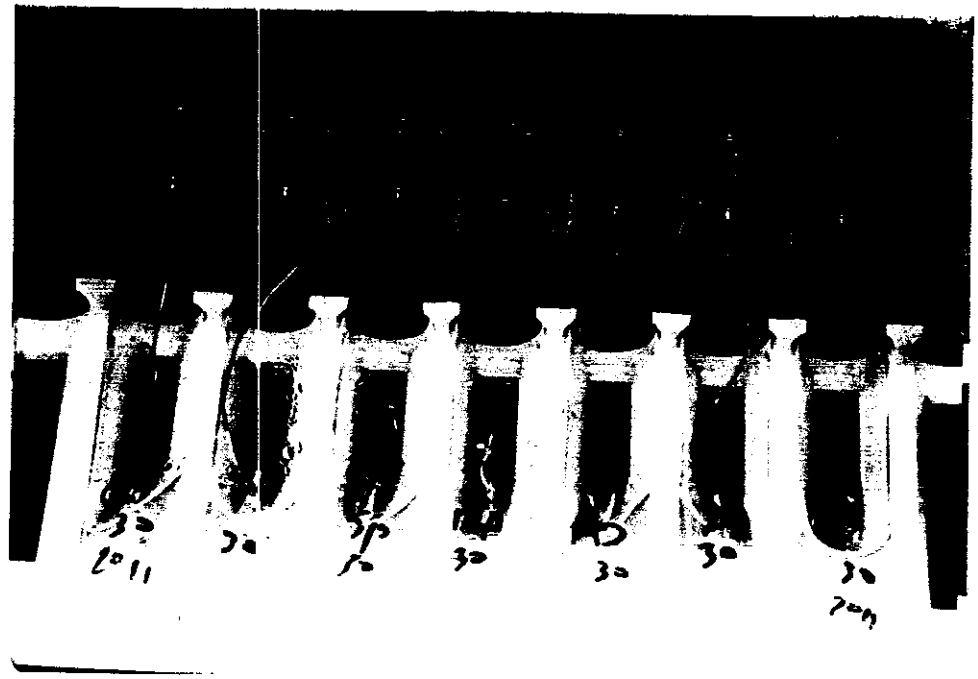


Untreated "Control"

F A C Y DA D S

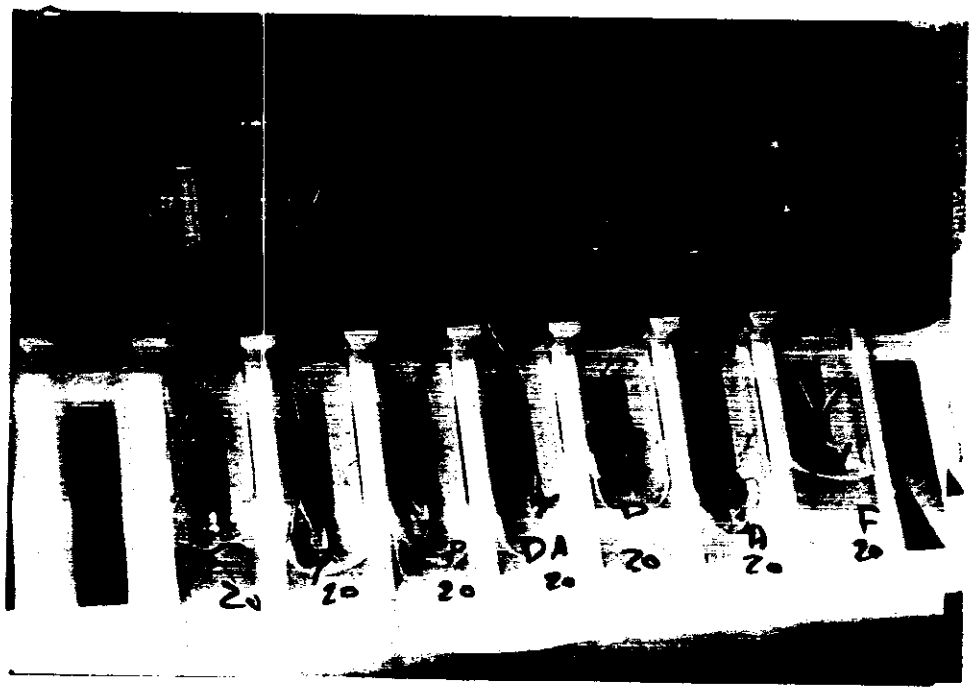
Different varieties

Fig. (8): Effect of different concentrations of thiouracil on the growth of nodal segments of different potato varieties. (F = Foundal; A = Alpha, C = Cara; Y - Jaerla; DA = diamont; D = Desiree and S = Spunta).



Thiouracil at 30 ppm

C Y SP DA D A F



Thiouracil at 20 ppm

C Y SP DA D A F

Different varieties

Fig. (8): Effect of treatment with thiouracil (continued).

individual varieties, all exhibited thiouracil effect on their growth values which significantly decreased than their untreated controls. From Table (10) and Fig.(8) could be observed that nodal segment cultures of Foundal, Desiree and Diamont varieties appeared to be slightly more sensitive to thiouracil-treatment especially at 30 ppm concentration. However, Alpha variety shows the lowest values of relative growth at all tested concentrations.

b. On inviability:

Apical meristem tips 1.00 mm long were excised from shoots developed on nodal segments during chemotherapy treatment. The excised tips were cultured on thiouracil-free nutrient MS medium and incubated for 5 weeks as usual. Then the numbers of inviable cultures as a result of thiouracil treatment were obtained.

Data summarized in Table (11) showed that the number of inviable cultures originated from thiouracil-treated ones, regardless potato varieties, was higher than those taken from untreated control. The latter gives 25.6% mortality, however, the correspondings were 40.8%, 44.9% and 49.7% for thiouracil-treatment at the rates of 10, 20 and 30 ppm, respectively. With pointing to varieties, the same trend was also noted. In general, the higher thiouracil concentrations resulted in greater mortality of apical meristem tip cultures, especially with varieties Alpha, Diamont and Foundal, which were the most sensitive to thiouracil treatment than the other varieties. In this respect, we must remember that the phytotoxicity was also

Table (11): Effect of thiouracil-treatments on the numbers of inviable shoot tip(1.0 mm long) cultured for different potato varieties.

Varieties	No. of tips used for each conc.	Number of inviables at different thiouracil concentrations (in ppm) 0	10	20	30	Total	%
Alpha	48	16	20	23	26	85/192*	44.3
Caro	48	10	22	22	24	78/192	40.6
Diamont	48	13	22	24	24	83/192	43.2
Desiree	48	9	18	18	21	66/192	34.4
Foundal	48	13	21	21	24	79/192	41.1
Jaerla	48	12	17	22	26	77/192	40.1
Spunta	48	13	17	21	22	73/192	38.0
Total	336	86	137	151	167	541/1344	-
%		25.6	40.8	44.9	49.7	40.3	

* Total inviable cultures which died without contamination symptoms/total shoot tips cultured.

observed earlier (Fig. 9) in nodal segment cultures treated with 30 ppm of thiouracil, in addition death of all cultures treated with 50 ppm or more.

c. On survivals and plantlets development:

Data in Table (12) showed that survivals were greatly reduced with thiouracil-treatment. The reduction was successfuly increased with increasing thiouracil concentrations. Of total 1344 apical meristem-tip cultures, only 678 were survived (50.4%). With regarding thiouracil levels, out of 336 cultures for each, 228 (67.9%), 169 (50.3%), 145 (42.2%) and 136 (40.5%) were survived for 0 (untreated), 10, 20 and 30 ppm, respectively. As for potato varieties, regardless thiouracil concentrations, the number of survivals were slightly differed, it was ranged between 90-108 out of 192 apical meristem tip cultures for each variety. Desiree and Spunta varieties were the best in this respect. For individual varieties, survivals were clearly decreased with increasing thiouracil-concentrations.

With regarding thiouracil-effect on the growth and developing into plantlets the same trend of the above results was observed. Out of 228, 169, 145 and 136 cultures survived at 0 (untreated), 10, 20 and 30 ppm of thiouracil, 151 (66.2%), 93 (55.0%), 83 (57.2) and 75 (55.1%) developed into plantlets, respectively. With varietal comparisons, the varieties Desiree and Fcundal resulted in the highest and lowest numbers of plantlets, respectively, but Cara variety gives the highest percentage (72.6%) in this respect. The plantlet-growth in

Table (12): Numbers of developed plantlets/numbers of survivals for different potato varieties as affected by different concentrations of thiouracil.

Varieties	No. of cultures used for each conc.	Thiouracil concentrations (in ppm)				Total	%
		0	10	20	30		
Alpha	48	20/32	8/23	8/17	9/18	45/90 *	50.0
Cara	48	21/34	14/19	20/22	14/20	69/95	72.6
Diamont	48	24/33	12/23	16/18	12/16	64/90	71.1
Desiree	48	24/35	20/28	12/23	16/22	72/108	66.7
Foundal	48	18/29	7/22	7/22	4/20	36/93	38.7
Jaerla	48	20/32	22/26	10/22	8/18	60/98	61.2
Spunta	48	24/33	10/28	10/21	12/22	56/104	53.8
Total	336	151/228	93/169	83/145	75/136	402/678	-
%		66.2	55.0	57.2	55.1	59.3	-

* Number of plantlets that developed/number of cultures that survived.

Cara and Jaerla varieties was the best of all tested varieties treated with thiouracil. The in vitro growth of such two varieties was enhanced on media containing 30 ppm of thiouracil, while the growth of other varieties were greatly supressed (Fig. 8) and Table (10).

d. On production of PVX-free plantlets:

Data summarized in Table (13) showed that any of the different levels of thiouracil treatment resulted in increasing number and percentage of PVX-free plantlets. These were successfuly increased with increasing of thiouracil concentrations. Of total of 176 virus-free plantlets obtained 36, 43, 48 and 49 of them were arised on media containing thiouracil at 0, 10, 20 and 30 ppm, respectively. The corresponding percentages of such numbers of virus-free plantlets were 23.8%, 46.2%, 57.8% and 65.3%, such wide range could be attributed to the above mentioned reduction in plantlets number which occurred as a result of thiouracil treatment.

As regards potato varieties, they could be arranged in descending order according to number of virus-free plantlets as Desiree, Cara, Jaerla, Diamont, Spunta, Foundal and Alpha. The number of virus-free plantlets was ranged between 16 to 40 for such different varieties. With the different concentrations of thiouracil, such varieties were also reacted differently. The highest numbers of virus-free plantlets were obtained at 10 ppm of thiouracil for Diamont (7/12) and Jaerla (8/22); at 20 ppm for Cara (13/20) and at 30 ppm for Desiree (13/16) and Spunta

Table (13): Effect of treatment with different concentrations of thiouracil on elimination of PVX*, the number of PVX-free (a) and total developed plantlets (b) for different potato varieties.

Variety	Thiouracil concentrations (in ppm)								Total	
	0		10		20		30			
	a/b	%	a/b	%	a/b	%	a/b	%	a/b	%
Alpha	4/20	20.0	3/8	37.5	4/8	50.0	5/9	55.6	16/45**	35.6
Cara	6/21	28.6	7/14	50.0	13/20	65.0	9/14	64.3	35.69	50.7
Diamont	5/24	20.8	7/12	58.3	6/16	37.5	6/12	50.0	24/64	37.5
Desiree	7/24	29.2	11/20	55.0	9/12	75.0	13/16	81.3	40/72	55.6
Foundal	5/18	27.8	3/7	42.9	5/7	71.4	4/4	100.0	17/36	47.2
Jaerla	4/20	20.0	8/22	36.4	7/10	70.0	6/8	75.0	25/60	41.7
Spunta	5/24	20.8	4/10	40.0	4/10	40.0	6.12	50.0	19/56	33.9
Total	36/151		43/93		48/83		49/75		176/402	
%	23.8		46.2		57.8		65.3		43.8	

* at the last indexing test i.e. after 10 weeks from potting.
** Total plantlets freed from PVX/total plantlets obtained.

(6/12). However, in Alpha and Foundal varieties, the numbers of PVX-free plantlets seemed to be unchanged, if not decreased with the thiouracil treatment. In such two latter varieties, as well as in other ones, the highest percentages of obtained healthy plantlets were associated with thiouracil-treatment at the highest concentrations i.e 30 ppm. In Foundal variety only 4 plantlets were obtained at such concentration, all of them were free from the virus.

From the above explanations of our results we can conclude that, using of thiouracil through in vitro culturing as antiviral agent for elimination of PVX from apical meristem tip cultures was effective when used at concentrations ranging from 10 to 30 ppm. However, at 50 ppm or more, it was harmfull and phytotoxic as all treated cultures were quickly dead. Successful elimination of the concerned virus was largely depending on potato variety and thiouracil concentrations used.

Effect of etiolation-treatment :

This experiment was conducted to estimate the in vitro growth which may be resulted in longest apical portions free from potato virus X. For this aim, in vitro plantlets were divided into nodal segments, as previously described (Material and Methods), cultured on nitrient MS medium with or without additional GA_3 at rate of 1.0 mg/l, then incubated for 2 weeks under complete darkness. The different effects of such treatments compared with control one (incubation was carried under continuous light) could be dealt as follows:

a. On length of shoot and number of roots:

Data in Table (14) showed that nodal segment cultures incubated under complete darkness either in the presence or absence of GA_3 resulted in the longest growing shoots and mostly the largest number of developed roots. In this respect, cultures incubated under continuous light showed the reverse order, in which both shoot elongation and root formation were slightly suppressed.

As for potato varieties incubation under complete darkness combined with addition of GA_3 to the culture media resulted in the longest etiolated shoots and largest numbers of developed roots in most varieties (Fig. 9). However, in variety Jaerla, root formation was completely inhibited, but in varieties Spunta and Desiree was the reverse. Addition of GA_3 seems to be not affected on root formation in variety Foundal.

b. On inviability:

Apical meristem portions 1.0 mm long were taken from shoots grown onto the above mentioned treatments, cultured and incubated as usual for 5 weeks the number of inviable cultures were counted and calculated. Results were illustrated in Table (15). From data in such Table it could be noted that GA_3 associated with complete darkness greatly reduced number of inviable cultures (13.4%). In this respect, incubation in darkness without GA_3 addition was the next (19.9%). The most inviability was in cultures ircubated under continuous light (23.2%). As for potato varieties, the same trend of results was observed. The

Table (14): Means of length (in mm) of regrowing shoot(s) and number of roots per nodal segment affected by etiolation treatments for different potato varieties.

Variety	Mean of shoot length (mm) at			Mean	Mean of root number at			Mean
	Darkness treat.	Darkness + GA_3 treat.	Cont. light treat.		Darkness treat.	Darkness + GA_3 treat.	Cont. light treat.	
Alpha	80	88	69	79.0	4.8	6.2	5.2	5.4
Cara	71	77	56	68.0	5.0	6.2	2.8	4.7
Diamont	72	83	67	74.0	6.0	6.5	4.0	5.5
Desiree	72	90	61	74.3	5.1	7.0	3.8	5.3
Foundal	62	77	35	58.0	5.4	5.4	2.6	4.5
Jaerla	59	68	43	56.7	3.6	0.0	1.8	1.8
Spunta	52	89	40	60.3	4.5	8.8	3.5	5.6
Mean	66.9	81.7	53.0	-	4.9	5.7	3.4	-

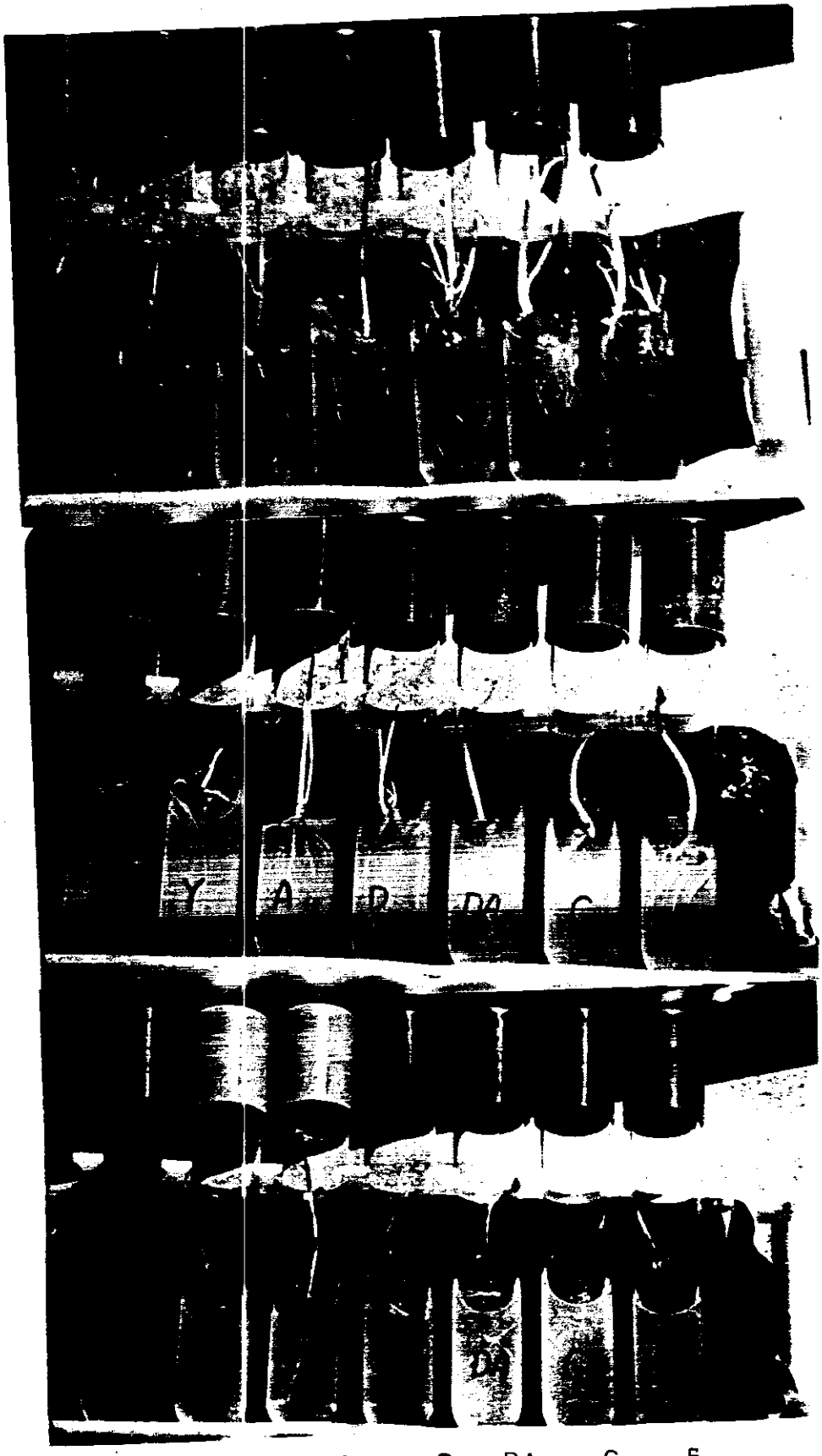


Fig. (9): Effect of continuous light and darkness with or without additional GA_3 on in vitro shooting, shoot length and rooting of nodal segments of different potato varieties.

Table (15): Culture inviability of excised shoot tips as affected by etiolation treatment compared with continuous light.

Variety	No. of cultures/ each treatment	Numbef of inviable cultures at etiolation treatments (i.e.) Darkness	Darkness + GA_3	Light	Total	%
Alpha	48	13	8	11	32/144*	22.2
Cara	48	8	5	17	30/144	20.8
Diamont	48	10	4	8	22/144	15.3
Desiree	48	10	6	8	24/144	16.7
Foundal	48	8	6	14	28/144	19.4
Jaerla	48	10	9	12	31/144	21.5
Spunta	48	8	7	8	23/144	16.0
Total	336	67	45	78	196	19.4
%	100.0	19.9	13.4	23.2	19.4	

* Total number of shoot tips inviable/total excised.

addition of GA_3 was most effective in the varieties Cara and Foundal, but not in Spunta.

c. On survivals and developed plantlets:

Data in Table (16) indicated that cultures incubated at either continuous light or darkness + GA_3 regardless varieties, resulted in equal numbers of survivals. Out of 336 cultures 208 (61.9%) were survived for both treatments. But these were increased to 220/336 (65.5%) in case of incubation under darkness without GA_3.

With regarding growth into plantlets, more plantlets developed under light condition i.e. 82.2% (171/208) compared with 57.2% (119/208) and 66.8% (177/220) for incubation under complete darkness with or without GA_3, respectively.

In general, the percentages of developed plantlets ranged between 60.0-90.9%, 41.2-69.0% and 53.6-78.1% for incubation under continuous light or complete darkness with or without GA_3, for all tested potato varieties, respectively. Among different varieties, Cara and Foundal produced the highest (75.0%) and lowest (52.7%) percentages of developed plantlets, however, variety Spunta resulted in the largest number i.e. 69 out of 96 survivals were developed into plantlets.

d. On elimination of PVX:

Data of Table (17) proved that 22.4% i.e 98 out of 437 plantlets could be freed from PVX by using this study. Regardless potato varieties, relatively equal percentages of virus-free plantlets i.e 24.4% and 23.8% were obtained from cultures

Table (16): Effect of preincubation under continuous light or darkness with or without additional GA_3 in culture medium on survivals and developed plantlets.

Variety	No. of cultures/ each treatment	Etiolation treatment			Total	%
		Dark-ness	Dark-ness + GA_3	Cont. light		
Alpha	48	15/28*	16/23*	28/31*	59/82*	72.0
Cara	48	25/32	18/30	20/22	63/84	75.0
Diamont	48	24/32	20/32	25/32	69/96	71.9
Desiree	48	23/33	15/32	29/33	67/98	68.4
Foundal	48	19/32	14.34	15/25	48/91	52.7
Jaerla	48	19/33	18/30	27/31	64/94	68.1
Spunta	48	22/30	18/27	27/34	67/91	73.6
Total	336	147/220	119/208	171/208	437/636	68.7
		66.8	57.2	82.2	68.7	

* Number of plantlets that developed out of the total shoot tips that survived.

incubated under complete darkness either with or without addition of GA_3 to culture media. In this respect, cultures grown under continuous light resulted in lowest percentage of healthy plantlets i.e. 19.9%. On the other hand, addition of GA_3 associated with complete darkness caused reduction in the number of virus-free plantlets . Out of 119 plantlets 29 only were healthy. The corresponding ones were 34/171 and 35/147 for incubation under continuous light or darkness without GA_3, respectively.

As for potato varieties, percentages of virus-free plantlets were ranged between 14.5%-30.2% for different varieties, the lowest and highest figures were for Cara and Diamont, respectively. Incubation under darkness without GA_3 and continuous light was superior for Cara and Jaerla varieties, as 8 plantlets out of 25 and 27 could be freed from PVX, respectively. For variety Alpha, the number of healthy plantlets was not improved i.e only 3 were obtained by using any of tested treatment.

From such results it could be concluded that GA_3 added to culture media at the rate of 1.0 mg/l combined with incubation under complete darkness was not an effective method for increasing number of either total plantlets or even those virus-free. On the other hand, incubation under complete darkness without GA_3 was beneficial in this respect.

Potato propagation through in vitro tuberization and micro-tuber productivity as affected by PVX-elimination:

An experiment was designed for investigating the available success to propagate potato varieties Cara Spunta and Foundal through in vitro tuberization and also to estimate comparable productivity of microtubers and their size frequency as affected by elimination of PVX from plant materials used. Results were tabulated and photographed in Table (18) and Figs. (10, 11, and 12). These could be summarized as follows:

Cara variety:

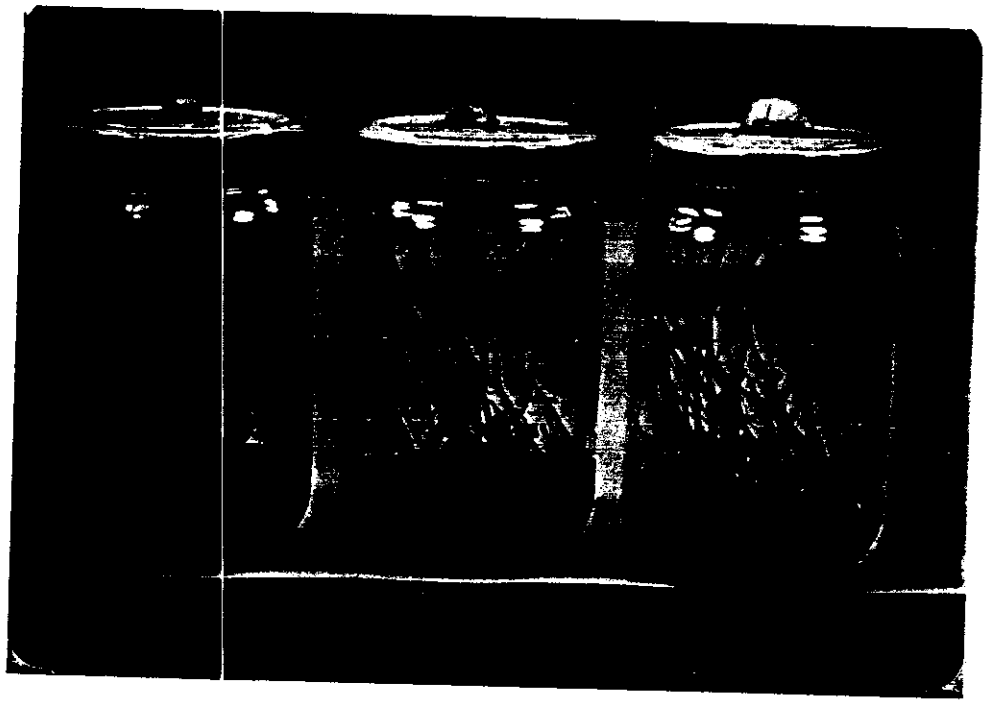
Data presented in Table (18) showed that the number of microtubers harvested as well as weight and diameter of the individual microtubers greatly varied between the different vials (replications) used for either PVX-free (healthy) or infected cultures. It could be noted that also, the values represents all of these measurements were higher in healthy than infected cultures. For healthy cultures, 12.3 microtubers could be obtained from each vial with an average weight 189.5 mg/microtuber and 5.8 mm diameter. The correspondings were 7.8, 73.8 mg and 2.4 mm for infected cultures, respectively. On other means elimination of PVX from cultures used for in vitro tuberization resulted in an increase of mean number of harvested microtuber/vial, means of weight and diameter of single microtubers by 57.7%, 156.8%, and 141.7% more than cultures still infected with such virus, respectively.

Table (18): In vitro micotubers productivity of PVX-free plantlets (Healthy) compared to those of infected ones for different potato varieties.

Varieties	Cara variety						Spunta variety						Foundal variety					
Serial number of vials	Number of microtubers/ vial		Mean weight of microtubers/ vial (mg)		Mean diameter of microtuber/ vial (mm)		Number of microtubers/ vial		Mean weight of microtubers/ vial (mg)		Mean diameter of microtuber/ vial (mm)		Number of microtubers/ vial		Mean weight of microtubers/ vial (mg)		Mean diameter of microtuber/ vial (mm)	
	H.*	Inf.*	H.	Inf.	H.	Inf.	H.	Inf.	H.	Inf.	H.	Inf.	H.	Inf.	H.	Inf.	H.	Inf.
1	10.0	7.0	123.0	101.0	5.3	3.0	13.0	13.0	162.0	94.0	5.10	4.00	6.0	5.0	208.0	88.0	4.20	3.40
2	21.0	9.0	131.0	78.0	5.0	2.9	12.0	11.0	133.0	61.0	4.30	2.00	12.0	4.0	183.0	95.0	4.80	3.10
3	16.0	10.0	198.0	62.0	5.8	1.4	5.0	11.0	222.0	80.0	5.60	1.70	6.0	2.0	202.0	98.0	4.80	3.40
4	15.0	4.0	155.0	58.0	5.5	2.5	8.0	14.0	254.0	58.0	6.00	2.60	7.0	3.0	174.0	87.0	5.00	3.80
5	9.0	5.0	257.0	84.0	6.8	2.6	15.0	9.0	243.0	69.0	5.10	2.40	5.0	3.0	182.0	122.0	4.70	4.00
6	8.0	12.0	249.0	60.0	6.4	1.9	14.0	12.0	224.0	95.0	5.10	4.50	6.0	-	167.0	-	3.40	-
7	8.0	-	209.0	-	6.6	-	14.0	6.0	132.0	85.0	4.80	2.50	5.0	-	124.0	-	5.00	-
8	11.0	-	194.0	-	5.1	-	-	-	-	-	-	-	-	-	-	-	-	-
Mean	12.3	7.8	189.5	73.8	5.8	2.4	11.6	10.9	195.7	77.4	5.14	2.81	6.7	3.4	177.1	98.0	4.56	3.54
Difference** %	+57.7		+56.8		+41.7		+6.4		+152.8		+82.9		+97.1		+80.7		+28.8	

* H = Healthy, Inf. = Infected.

** Difference % = Healthy-infected/infected (100).



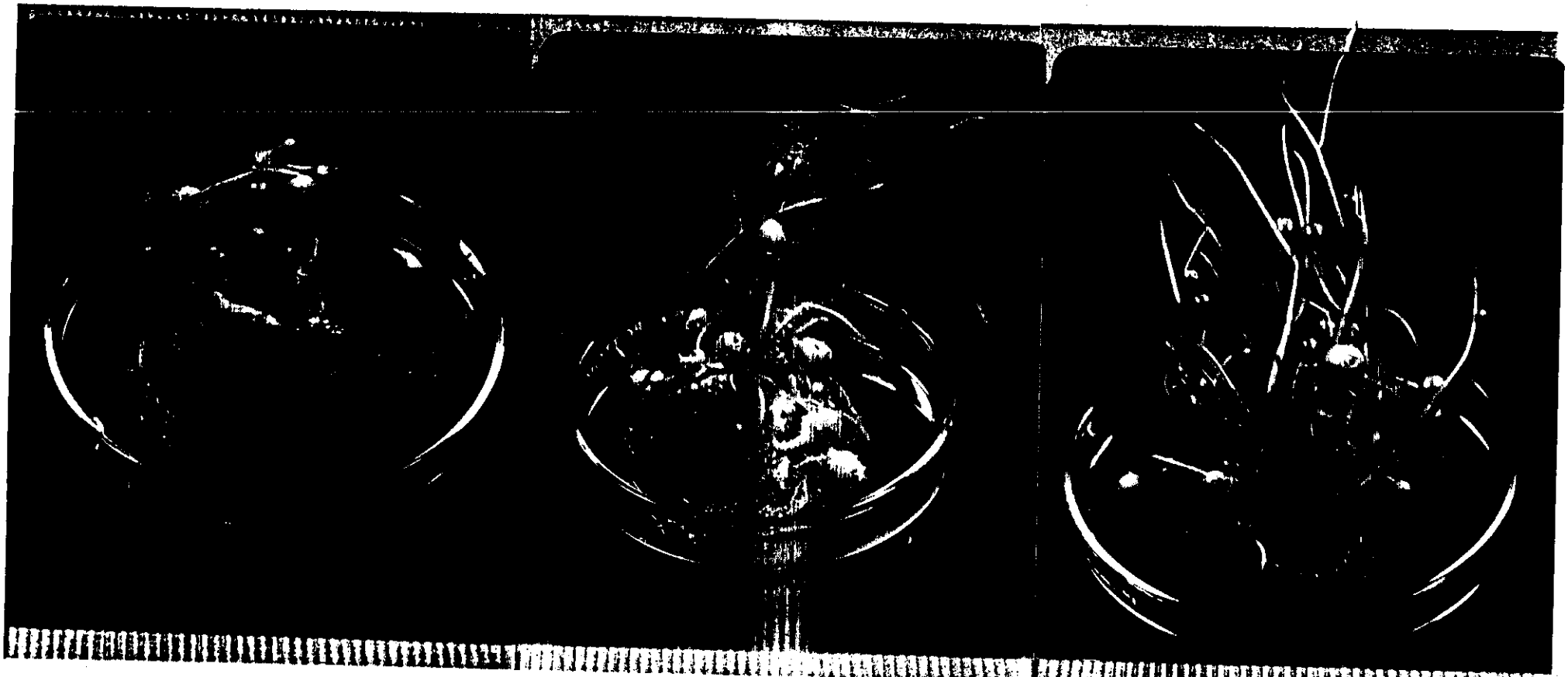
PVX-free cultures

PVX-infected cultures

Foundal Spunta Cara

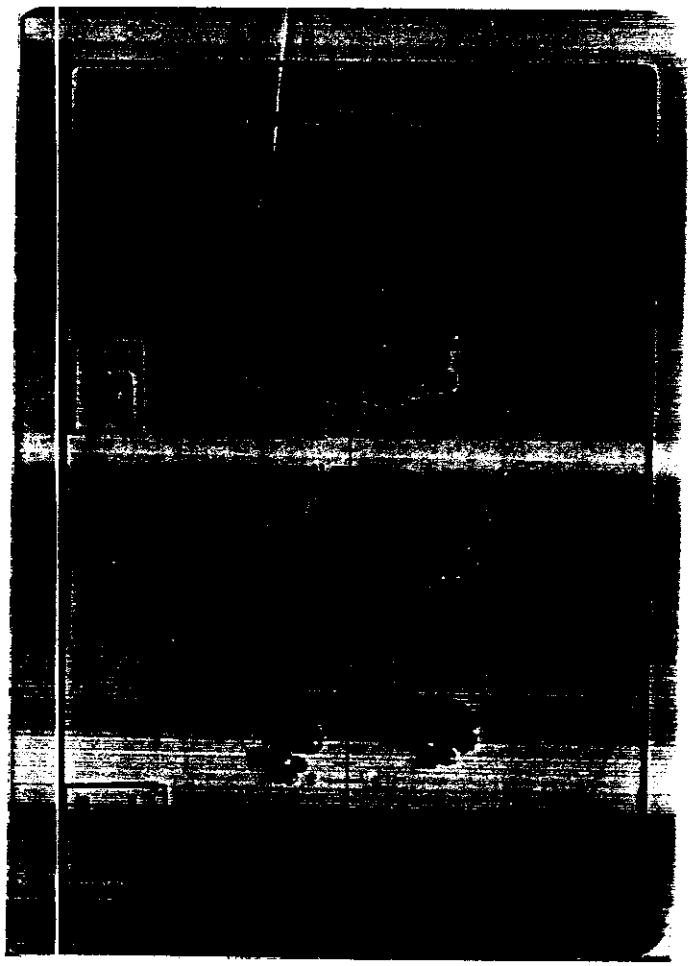
Different varieties

Fig. (10): In vitro tuberization from PVX-free and infected potato nodal segment cultures, one week before harvesting.



Foundal var. Spunta var. Cara var.

Fig. (11): In vitro potato microtubers, on the time of harvesting. All resulted from PVX-free cultures.



From Cara variety

From Spunta variety

From Foundal variety

PVX-infected

PVX-infected

Virus status

Fig. (12): Effect of PVX-elimination on the number and size of in vitro micro-tuber for three potato varieties (Right = PVX-free (healthy) culture) (Left = PVX-infected culture).

Spunta variety:

Data in Table (18) showed the same trend as above seen in Cara variety. However, using cultures free from PVX will be gained number of microtubers increased by 6.4% only over than those from infected ones. On the other side, both means of weight and diameter/microtuber were increased by 152.8% and 82.9% in healthy than infected cultures, respectively.

Foundal variety:

Results in Table (18) were paralel to those of Cara and Spunta varieties. In Foundal variety, using culture free from PVX for producing microtubers under in vitro conditions will be increased the mean number of microtubers/vial, mean weight and diameter of a single microtuber by 97.1%, 80.7% and 28.8%, respectively when compared with the yields obtained from cultures infected with this virus.

Effect of PVX-elimination on size frequency of the obtained microtubers:

Results summarized in Table (19) showed clearly that PVX-elimination could be improved size of the obtained *in vitro* microtubers for all tested potato varieties. In case of healthy cultures of Cara variety, out of 98 microtubers obtained 79 were equal to or larger than 5 mm diameter (80.6%). Only 2 microtubers of that size out of 47 were obtained from infected cultures. As for Spunta varieties 66.7% in opposite to 17.1% of the microtubers harvested from healthy and PVX-infected cultures, respectively were equal to or larger than

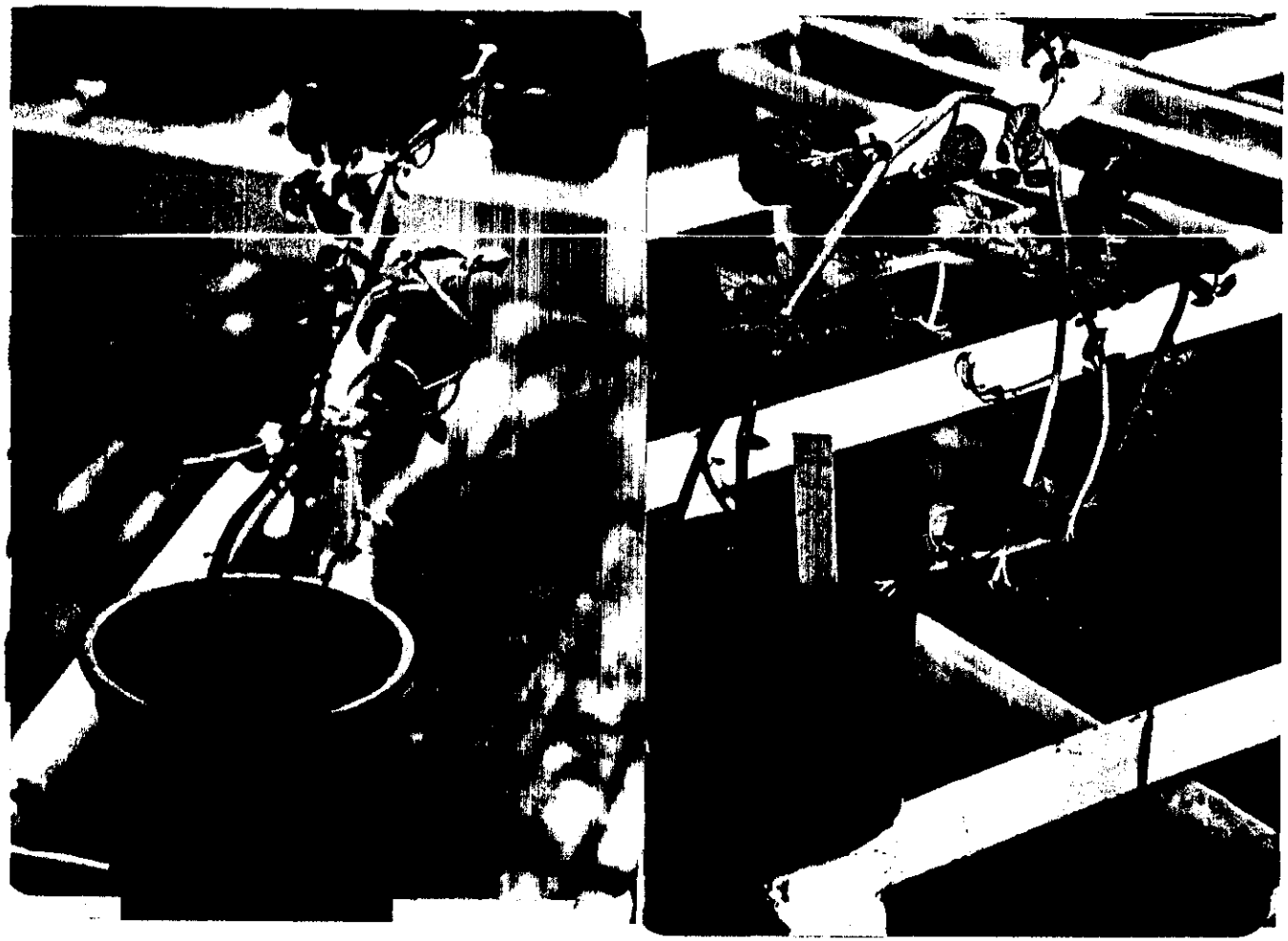
Table (19): Diameter frequency of in vitro microtubers obtained from PVX-free (Healthy) and infected potato varieties Cara, Spunta and Foundal.

Microtuber diameter equal to or more than (mm)	Number of microtuber for different potato varieties					
	Cara		Spunta		Foundal	
	H.*	In.*	H.	In.	H.	In.
1 mm	98	47	81	76	47	17
2 mm	96	31	79	58	42	16
3 mm	93	19	73	41	38	14
4 mm	90	8	66	25	34	8
5 mm	79	2	54	13	22	4
6 mm	56	-	38	6	11	-
7 mm	32	-	15	-	8	-
8 mm	12	-	3	-	3	-
9 mm	-	-	-	-	-	-
10 mm	-	-	-	-	1	-
5 mm** %	80.6	4.3	66.7	17.1	46.8	28.5
6 mm** %	57.1	0.0	46.9	7.9	23.4	0.0

* H = Healthy

In = Infected

** Equal to or larger than.



PVX-infected

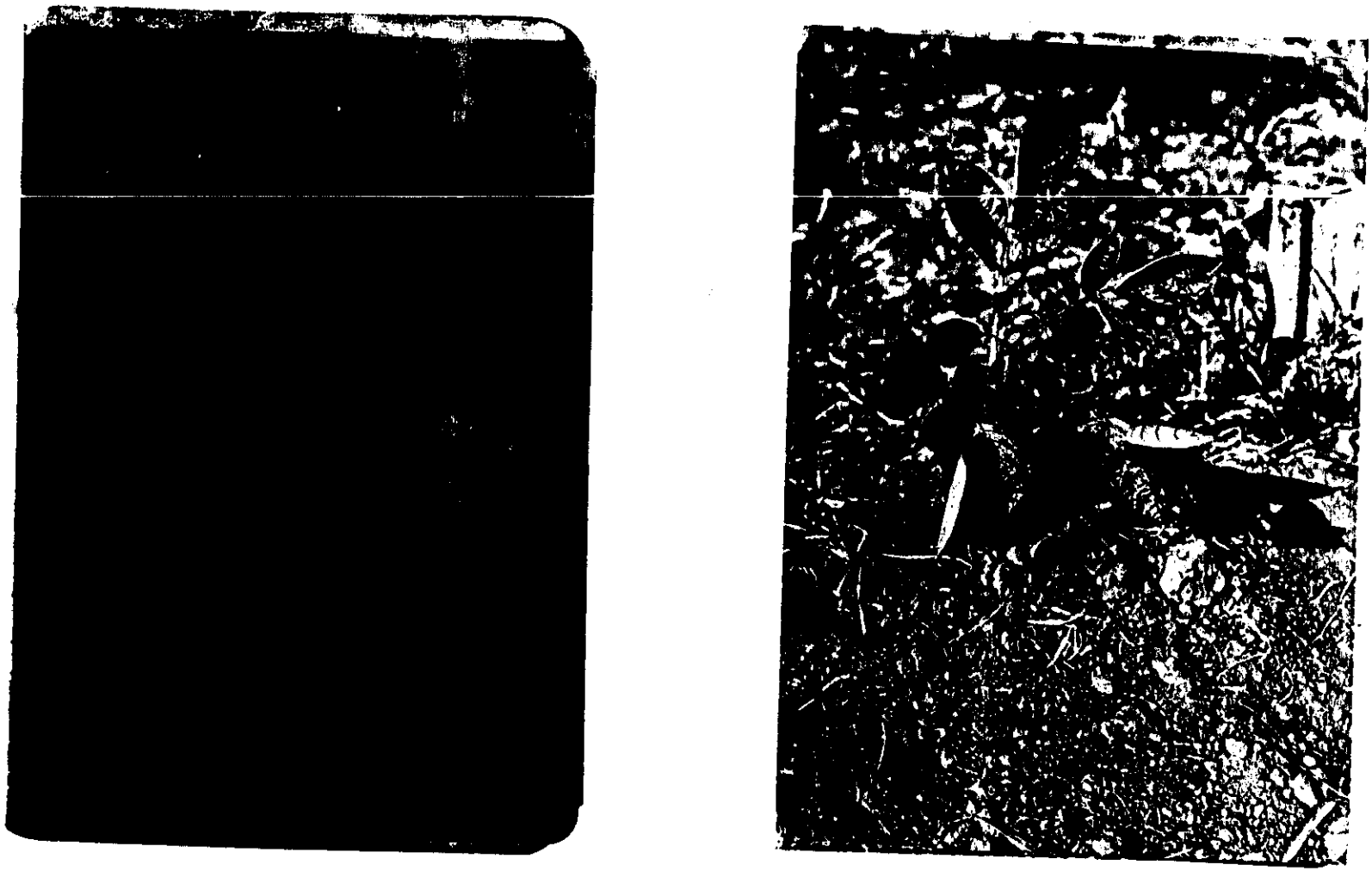
PVX-free

Fig. (13): Potato plants produced from in vitro microtubers, 2 months-old.

5 mm diameter with regarding PVX-free and infected cultures of Foundal variety, out of 47 and 17 microtubers 22 and 4 of the above selected size were obtained, respectively. In all cultures infected with PVX for any of the tested varieties, microtubers equal to or larger than 7 mm diameter could not be produced at all. However, in healthy cultures, these were 32/98, 15/81 and 8/47 for Cara, Spunta and Foundal varieties, respectively.

Indexing of microtubers for PVX-infectivity:

Undormant microtubers produced in vitro from either previously ascertained PVX-free or infected cultures from the above mentioned three potato varieties (Fig. 12) were germinated in potted soil mixture. After 10 weeks from planting, rub transmission test on leaves of Gomphria globosa plants using sap from growing plants emphasized the virus freedom of microtubers produced from healthy cultures. All microtubers obtained from PVX-infected cultures resulted in infected growing plants. (Fig. 13). Potato plants from both types of microtubers were left to grow in pots for more than 2 months. In the healthy plant, no viral disease symptoms were appeared even plants were ageing up to more than 4 months, in contrast, symptoms of mild mosaic which progressed latter to severe mosaic began to appear after few weeks from planting of progeny tubers of the natural infected plant (Fig. 14).



Healthy

Infected

Fig. (14): PVX-free (healthy) and infected 3 months old plants from Cara variety originated from PVX-free and infected microtubers, respectively.