

Physiological Studies on Drought Stress Tolerance of Gamma Irradiated Grape Rootstocks Using Tissue Culture

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Abstract: *In vitro* cultured plantlets of two grape rootstocks, Freedom and SO₄ were exposed to Gamma rays at 0, 5, 10, 20, 30 and 40 Gray. Irradiated plantlets were sub cultured on PEG 6000 containing medium at 0, 0.5, 2, 4, 6, 8 and 10 g/L to evaluate their susceptibility to drought. Results of this study indicated that Gamma rays changed the characters of the two studied rootstocks. The Gamma dose of 40 Gray was lethal for both rootstocks plantlets. Similarly, the PEG concentration of 10 g/L dehydrated the plantlets as they couldn't survive more than a week. The 5 Gray dose seem to be a promoter for survival percentage, number of leaves/plantlet, average leaf area, number of roots/plantlet and average root length. The 10 Gray dose shared or succeeded the 5 Gray dose results in survival and most of growth parameters, particularly when combined with the lower PEG concentration of 0.5 g/L or under dehydration conditions. The Gamma dose of 20 Gray recorded low survival and growth parameters, except for a considerable increase in roots formation. However, the Gamma dose of 30 Gray had inhibited survival and all growth parameters when cultured on different media. Both rootstocks biologically had the same trend under all treatments even if SO₄ was superior in root length, while Freedom scored a greater number of roots.

Key words: Gamma irradiation • Micropropagation • Drought • PEG • Grape

INTRODUCTION

Grape (*Vitis vinifera*) is one of the most important commercial fruits and the second fruit crop in production after citrus in Egypt. Recently, grape area extended greatly in reclaimed regions. Survival of new varieties depends greatly on suitable rootstocks tolerant to local conditions. The high percentage of Gamma-ray irradiated mutants indicates that mutation breeding via Gamma-ray irradiation is an effective and highly successful approach for the generation of commercial cultivars [1]. Patil and Patil [2] studied gamma rays mutagens effects on Anab-e-Shahi grape and found that survival of mutants was higher at shorter duration of mutagenic treatments. The mutants recorded the highest number of leaves. Tayyar *et al.* [3] irradiated Amasya grape with the gamma doses 20, 25, 30 and 35 Gray and showed that vigour values were decreased with increasing radiation doses. Number of roots and rooting percentage of 20 Gray irradiated plants were declined. Harb *et al.* [4] studied the effects of PEG 0, 3, 6, 9, 12 and 15% and gamma irradiation 0, 10, 20, 30, 40, 50 and 60 Gray, on *in vitro* micropropagated banana and

pointed out that the gradual increase in PEG was negatively correlated with survival percentage, leaf and root number. Exposure to gamma irradiation 10 and 20 Gray alone or prior to PEG had stimulative effects on the growth. Higher doses of irradiation (30 and 40 Gray) adversely affected all the tested parameters. Gamma radiation higher than 50 Gray was lethal. Patade *et al.* [5] maintained *in vitro* mutagenesis to select drought tolerant lines in sugarcane. Embryogenic callus cultures were subjected to gamma rays at different doses. They were exposed to inhibitory levels of PEG 8000, 0.625, 1.25, 2.5, 3.75 and 5.00 mM. Plantlets selected on 0.625mM PEG accumulated maximum genetic changes.

The present study dealt with the effect of Gamma rays doses on Freedom and SO₄ rootstocks specifically their drought tolerance and testing them on PEG media.

MATERIALS AND METHODS

This work was carried out in the Tissue Culture Laboratory, Horticulture Research Institute, Agricultural Research Center and Middle East Regional Radioisotope

Center for Arab Countries, Giza, Egypt during the period from 2008 to 2011. This study aimed to induce and propagate an *in vitro* drought resistant mutagens of two grape rootstocks, Freedom (1613x *Vitis champini*) and SO₄ (*Vitis berlandieri* x *Vitis riparia*). Shoot tips (1cm length) of the two rootstocks transplants, which aged 6- 12 month- old were used as the culture explants, have been collected and soaked for 3 minutes in Savlon solution (Chlorohexidine) plus drops of Tween 20 as a surfactant. Explantlets rinsed thoroughly under running tap water for 30 minutes, then sterilized under laminar flow hood conditions with Clorox (commercial bleach) 10% for 15 minutes. Six weeks- old plantlets were exposed to 0, 5, 10, 20, 30 and 40 Gray of Gamma rays using cobalt 60 source El-Sayed *et al.* [6]. Irradiated plantlets were subcultured on PEG 6000 supplemented media at 0, 2, 4, 6, 8 and 10 g/L to test their water efficiency tolerance according to Bor *et al.* [7] and Imanparast and Hassanpanah [8]. The experiment was laid out in split – split plot design with five replicates for each treatment and the replicate presented by five plantlets.

Data were recorded after 3-4 weeks of subculture on PEG containing media, the following measurements were taken: Plantlet survival percentage was calculated by the following equation:

$$\text{Survival \%} = (\text{Number of survived plantlets} / \text{Total number of planted plantlets}) \times 100$$

Also, number of leaves/ plantlet, average leaf area (cm²), number of roots/ plantlet and average root length (cm) were recorded.

Statistical Analysis: All obtained data were subjected to analysis of variances (ANOVA) according to Silva and Azevedo [9] using Assistat-Statistical Assistance Software. Separation of means among treatments was determined using New LSD test at 5% according to Snedecor and Cochran [10].

RESULTS AND DISCUSSION

Plantlet Survival Percentage: Data illustrated in Table 1 and Fig. 1 revealed that irradiating grape plantlets with 5, 10 and 0 Gray of Gamma rays had maintained the highest survival percentages (100, 100 and 94.44%), respectively without significant differences. However, the 20 Gray dose had lower survival (91.67%) and the 30 Gray dose resulted in the lowest significant survival percentage (85.18%). Similarly, Patil and Patil [2] had proved that gamma rays grape mutants survival was higher at shorter duration of mutagenic treatments.

Table 1: Effect of Gamma rays and PEG treatments on plantlet survival (%)

Treatments		Rootstock		
PEG (g/L)	Rad. (Gray)	SO ₄	Freedom	MEAN
0	0	100.00	100.00	100.00
	5	100.00	100.00	100.00
	10	100.00	100.00	100.00
	20	88.89	100.00	94.44
	30	77.78	77.78	77.78
Mean		93.33	95.56	94.44
0.5	0	100.00	100.00	100.00
	5	100.00	100.00	100.00
	10	100.00	100.00	100.00
	20	100.00	100.00	100.00
	30	100.00	100.00	100.00
Mean		100.00	100.00	100.00
2	0	88.89	100.00	94.44
	5	100.00	100.00	100.00
	10	100.00	100.00	100.00
	20	88.89	100.00	94.44
	30	55.55	100.00	77.78
Mean		86.67	100.00	93.33
4	0	88.89	100.00	94.44
	5	100.00	100.00	100.00
	10	100.00	100.00	100.00
	20	88.89	100.00	94.44
	30	55.55	100.00	77.78
Mean		86.67	100.00	93.33
6	0	88.89	88.89	88.89
	5	100.00	100.00	100.00
	10	100.00	100.00	100.00
	20	100.00	77.78	88.89
	30	77.78	100.00	88.89
Mean		91.11	93.33	92.22
8	0	77.77	88.89	83.33
	5	100.00	100.00	100.00
	10	100.00	100.00	100.00
	20	77.78	100.00	88.89
	30	88.89	77.78	83.33
Mean		91.11	93.33	92.22
MEAN		92.22	96.30	-
Mean values of PEG	0	92.59	96.30	94.44
	5	100.00	100.00	100.00
	10	100.00	100.00	100.00
	20	87.04	96.30	91.67
	30	81.48	88.89	85.18

LSD (PEG) =7.47 LSD (Rad.) =6.82 LSD (Rootstock) =4.31

LSD (PEGxRad.)=16.71

LSD (PEG x Rootstock) =10.57 LSD (Rad.xRootstock) =9.64

LSD (PEGxRad. X Rootstock) =23.62



Fig. 1: Effect of Gamma rays doses on Freedom and SO_4 rootstocks from left to right: 5, 10, 20 and 30 Gray, A= Freedom
B= SO_4



Fig. 2: Effect of PEG concentrations on Gamma irradiated Freedom and SO_4 plantlets from left to right: 0, 0.5, 2, 4, 6 and 8 g/L PEG, A= SO_4 B= Freedom

Concerning PEG concentrations, it was found that culturing plantlets on 0.5, 0, 2 and 4g/ L PEG media resulted in (100, 94.44, 93.33 and 93.33%) survival percentages, while culturing plantlets on 6 and 8 g/L PEG media reduced their survival percentage significantly to 92.22% for both concentrations. These results are in agreement with those obtained by Harb *et al.* [4].

Freedom and SO_4 rootstocks survival percentages did not differ significantly with different treatments (96.30 and 92.22%). As for the interaction between Gamma doses and PEG concentrations on both rootstocks, from Table 1 and Fig. 2, it could be concluded that all combinations did not affect survival percentage significantly since it was 100%, except for 20 and 30 Gray

Table 2: Effect of Gamma rays and PEG treatments on Number of leaves/ plantlet

Treatments		Rootstock		
PEG (g/L)	Rad. (Gray)	SO ₄	Freedom	MEAN
0	0	5.40	5.16	5.28
	5	5.73	5.58	5.66
	10	5.56	5.43	5.50
	20	5.13	5.12	5.13
	30	4.63	4.34	4.48
Mean		5.29	5.13	5.21
0.5	0	5.33	4.90	5.12
	5	5.62	5.55	5.58
	10	5.44	5.35	5.40
	20	5.10	4.88	4.99
	30	4.36	4.20	4.28
Mean		5.17	4.98	5.07
2	0	4.93	4.79	4.86
	5	5.19	4.95	5.07
	10	5.14	4.91	5.03
	20	5.02	4.77	4.90
	30	4.31	4.07	4.19
Mean		4.92	4.70	4.81
4	0	4.32	4.00	4.16
	5	4.64	4.23	4.44
	10	4.68	4.27	4.48
	20	4.45	3.99	4.22
	30	4.23	3.96	4.10
Mean		4.47	4.09	4.28
6	0	3.83	3.50	3.66
	5	4.00	3.67	3.84
	10	4.12	3.88	4.00
	20	3.77	3.44	3.60
	30	3.34	3.18	3.26
Mean		3.81	3.53	3.67
8	0	3.34	3.07	3.21
	5	3.51	3.33	3.42
	10	3.49	3.46	3.48
	20	3.34	3.23	3.29
	30	3.07	2.91	2.99
Mean		3.35	3.20	3.27
MEAN		4.50	4.27	-
Mean values of PEG	0	4.53	4.24	4.38
	5	4.78	4.55	4.67
	10	4.74	4.55	4.64
	20	4.47	4.24	4.35
	30	3.99	3.78	3.88

LSD (PEG) =0.03 LSD (Rad.) =0. 03 LSD (Rootstock) =0.02 LSD (PEGxRad.) =0.06

LSD (PEG x Rootstock) =0.04 LSD (Rad.xRootstock) = 0.04 LSD (PEG x Rad.xRootstock) =0.09

irradiated plantlets which cultured on 0 and 2g/L PEG containing medium, survival percentages were 77.77%. These findings are in accordance with those obtained by Patade *et al.* [5].

Number of Leaves / Plantlet: Data in Table 2 and Fig. 1 pointed that different Gamma doses of 5, 10, 0, 20 and 30 Gray significantly affected on Number of leaves / plantlet which graded (from 4.67, 4.64, 4.38, 4.35 and 3.88), respectively. These findings are in accordance with those reported by Harb *et al.* [4] on banana and Patil and Patil [2] on grape. Looking to the effect of PEG, it was noticed that each PEG increase in the medium from 0, 0.5, 2, 4, 6 to 8 g/L showed a significant decrease in number of leaves/plantlet as the following 5.21, 5.07, 4.81, 4.28, 3.67 and 3.27, respectively. In this respect, Harb *et al.* [4] pointed out that gradual PEG increase had negative effects. As for the rootstocks, there were no significant difference in No. of leaves/ plantlet between SO₄ and Freedom (4.50 and 4.27, respectively).

The interaction between Gamma doses and PEG concentrations, Table 2 and Fig. 2 cleared that 5 Gray irradiated SO₄ plantlets which were cultured on a PEG free medium had formed the highest significant number of leaves / plantlet (5.73), while 30 Gray irradiated Freedom plantlets which were cultured on 8 g / L PEG medium had formed the lowest significant number of leaves/ plantlet (2.91).

Average Leaf Area (cm²): Data presented in Table 3 and Fig. 1 indicated that the largest leaf area (1.15 cm²) was recorded with 5 Gray irradiated plantlets, followed by 10 Gray dose (1.13cm²) and 20 and 0 Gray doses (1.04 and 1.01cm², respectively), while the smallest leaf area (0.95cm²) was recorded with 30 Gray dose.

Concerning PEG concentrations effects, it was found that 0.5 g/L significantly maximized average leaf area (1.27cm²) followed by the PEG free medium (1.18 cm²) and 2 g/L PEG concentration (1.08cm²). Leaf area at 4 or 6 g/ L PEG concentrations were 0.99 and 0.98cm², respectively, while at 8 g/L PEG which had minimized leaf area sharply (0.84cm²). Similar results pointed out by Harb *et al.* [4]. As for SO₄ and Freedom rootstocks they had a significant difference between them in average leaf area (1.12 and 0.99 cm², respectively).

Concerning, the interaction between Gamma doses and PEG concentrations, Table 3 and Fig. 2 showed that the largest leaf area (1.46 and 1.42cm²) was obtained with SO₄ plantlets irradiated by using 5 or 10 Gray and cultured on 0.5 g/L PEG medium, while the lowest average leaf area (0.66 cm²) was recorded by using 30 Gray irradiated Freedom plantlets which were cultured on 8 g/ L PEG medium.

Table 3: Effect of Gamma rays and PEG treatments on leaf area (cm²)

Treatments		Rootstock		
PEG (g/L)	Rad. (Gray)	SO ₄	Freedom	Mean
0	0	1.16	0.99	1.08
	5	1.34	1.21	1.27
	10	1.32	1.23	1.27
	20	1.24	1.15	1.19
	30	1.12	1.00	1.06
Mean		1.24	1.11	1.18
0.5	0	1.29	1.11	1.20
	5	1.46	1.30	1.38
	10	1.42	1.27	1.35
	20	1.35	1.16	1.26
	30	1.23	1.04	1.14
Mean	1.35	1.18	1.27	
2	0	1.12	0.96	1.04
	5	1.27	1.15	1.21
	10	1.20	1.12	1.16
	20	1.13	0.99	1.06
	30	1.00	0.88	0.94
Mean		1.14	1.02	1.08
4	0	1.01	0.90	0.96
	5	1.14	0.93	1.04
	10	1.12	1.00	1.06
	20	1.07	0.93	1.00
	30	0.99	0.85	0.92
Mean		1.07	0.92	0.99
6	0	1.00	0.89	0.95
	5	1.13	0.93	1.03
	10	1.11	0.98	1.05
	20	1.00	0.93	0.97
	30	0.98	0.85	0.91
Mean		1.05	0.91	0.98
8	0	0.87	0.81	0.84
	5	0.99	0.91	0.95
	10	0.95	0.85	0.90
	20	0.84	0.76	0.80
	30	0.78	0.66	0.72
Mean		0.89	0.80	0.84
MEAN		1.12	0.99	-
Mean values of PEG	0	1.08	0.94	1.01
	5	1.22	1.07	1.15
	10	1.19	1.07	1.13
	20	1.10	0.99	1.04
	30	1.02	0.88	0.95

LSD (PEG) =0.02 LSD (Rad.) =0.02 LSD (Rootstock) =0.01 LSD (PEGxRad.) =0.04

LSD (PEG x Rootstock) =0.03 LSD (Rad.xRootstock) =0.03 LSD (PEG x Rad.xRootstock) =0.06

Table 4: Effect of Gamma rays and PEG treatments on Number of roots/plantlet

Treatments		Rootstock		
PEG (g/L)	Rad. (Gray)	SO ₄	Freedom	Mean
0	0	6.60	7.20	6.90
	5	7.00	7.33	7.17
	10	7.90	7.95	7.93
	20	9.77	9.72	9.75
	30	5.70	4.99	5.35
Mean		7.40	7.44	7.42
0.5	0	8.00	8.28	8.14
	5	8.78	8.77	8.77
	10	8.96	8.97	8.97
	20	10.00	9.94	9.97
	30	6.90	6.76	6.83
Mean		8.53	8.54	8.54
2	0	6.45	6.60	6.53
	5	7.29	7.23	7.26
	10	7.97	7.95	7.96
	20	8.94	8.98	8.96
	30	5.76	5.77	5.77
Mean		7.28	7.30	7.29
4	0	5.82	6.17	5.99
	5	6.30	6.35	6.33
	10	6.83	6.85	6.84
	20	7.87	7.78	7.83
	30	5.56	5.47	5.51
Mean		6.48	6.52	6.50
6	0	4.60	5.27	4.93
	5	5.19	5.30	5.24
	10	5.71	5.66	5.69
	20	6.30	6.25	6.28
	30	3.68	3.34	3.51
Mean		5.10	5.16	5.13
8	0	3.33	3.50	3.41
	5	3.95	3.83	3.89
	10	4.74	4.71	4.73
	20	5.58	5.45	5.51
	30	2.98	2.87	2.92
Mean	4.11	4.07	4.09	
MEAN	6.48	6.51		
Mean values of PEG	0	5.80	6.17	5.98
	5	6.42	6.47	6.44
	10	7.02	7.02	7.02
	20	8.08	8.02	8.05
	30	5.10	4.87	4.98

LSD (PEG) =0.02 LSD (Rad.) =0.02 LSD (Rootstock) =0.01 LSD (PEGxRad.) =0.04

LSD (PEG x Rootstock) =0.03 LSD (Rad.xRootstock) =0.02 LSD (PEG x Rad.xRootstock) =0.06

Table 5: Effect of Gamma rays and PEG treatments average root length (cm)

PEG (g/L)	Rad. (Gray)	SO ₄	Freedom	Mean
0	0	11.07	11.28	11.18
	5	12.95	12.55	12.75
	10	13.28	13.11	13.19
	20	8.00	7.86	7.93
	30	5.66	5.50	5.58
Mean		10.19	10.06	10.13
0.5	0	11.73	12.01	11.87
	5	13.01	13.12	13.07
	10	13.16	13.09	13.13
	20	9.55	9.32	9.44
	30	6.06	5.97	6.01
Mean		10.70	10.70	10.70
2	0	10.08	10.17	10.13
	5	12.50	12.49	12.50
	10	12.97	12.87	12.92
	20	6.83	6.32	6.58
	30	6.03	5.98	6.01
Mean		9.68	9.56	9.62
4	0	9.03	9.20	9.12
	5	11.87	11.87	11.87
	10	11.92	11.85	11.89
	20	4.89	4.87	4.88
	30	4.23	4.15	4.19
Mean		8.39	8.39	8.39
6	0	7.67	7.71	7.69
	5	11.74	11.67	11.71
	10	11.79	11.75	11.77
	20	4.78	4.68	4.73
	30	3.58	3.37	3.48
Mean		7.91	7.84	7.87
8	0	5.00	5.12	5.06
	5	9.64	9.49	9.56
	10	9.88	9.78	9.83
	20	3.40	3.17	3.28
	30	1.88	1.86	1.87
Mean		5.96	5.88	5.92
MEAN		8.81	8.74	-
Mean values of PEG	0	9.10	9.25	9.17
	5	11.95	11.87	11.91
	10	12.17	12.07	12.12
	20	6.24	6.03	6.14
	30	4.57	4.47	4.52

LSD (PEG) =0.02 LSD (Rad.) =0.02 LSD (Rootstock) =0.01 LSD (PEGxRad.) =0.03

LSD (PEG x Rootstock) =0.03 LSD (Rad.x Rootstock) =0.06 LSD (PEG x Rad.x Rootstock) =0.05

Number of Roots/ Plantlet: Data in Table 4 and Fig. 1 illustrated that 20 Gray of Gamma rays significantly increased Number of roots/ plantlet (8.05), followed by 10 or 5 Gray doses which scored (7.02 and 6.44,

respectively), while the non irradiated plantlets had 5.98 roots/plantlet with significant difference between all doses. However, the 30 Gray dose decreased roots number up to 4.98. On the contrary, Tayyar *et al.* [3] found that grapes irradiated with 20G gamma rays declined roots number compared to control, but no rooting was observed with the 30G dose.

Looking at PEG concentrations effects, it could be seen that the highest number of roots/ plantlet (8.54) was for 0.5g/L PEG medium. Root numbers was decreased gradually with increasing PEG in media of 0, 2, 4 and 6 g/L, as they were (7.42, 7.29, 6.50 and 5.13 respectively). The 8 g/ L PEG containing medium recorded the lowest Number of roots/ plantlet (4.09), with significant differences between various concentrations. These results are in harmony with those obtained by Harb *et al.* [4]. As for Freedom and SO₄ rootstocks, there was a significant difference between them (6.51 and 6.48 root/ plantlet, respectively).

Regarding the interaction between Gamma doses and PEG concentrations, Table 4 and Fig. 2 indicated that SO₄ plantlets which received 20 Gray irradiated and cultured on 0.5g/ L PEG medium increased Number of roots/plantlet up to 10 roots, whereas culturing 30 Gray irradiated SO₄ and Freedom rootstocks on 8 g/L PEG medium reduced Number of roots/ plantlet up to 2.98 and 2.87, respectively.

Average Root Length (cm): Data presented in Table 5 and Fig. 1 indicated that irradiating plantlets with the 10 Gray Gamma doses resulted in the tallest roots (12.12cm) followed 5 Gray (11.91cm) and non irradiated plantlets (9.17 cm), while the shortest roots (6.14 and 4.52 cm) were recorded with 20 and 30 Gray irradiated plantlets.

Concerning PEG concentrations, it was noticed that the medium with 0.5g/L gave the highest significant root length (10.70cm), while the length was decreased gradually by increasing concentrations from 0, 2, 4, 6 to 8 g/L as following (10.13, 9.62, 8.39, 7.87 to 5.92 cm), respectively with significant differences between concentrations. Regarding the rootstocks it was found that SO₄ had formed taller roots (8.81cm) than those of Freedom (8.74cm) with significant difference between them. As for the interaction between Gamma doses and PEG concentrations, Table 5 and Fig. 2, it could be concluded that the 10 Gray irradiated SO₄ plantlets cultured on PEG free medium recorded the tallest roots (13.28cm), while culturing 30 Gray plantlets of SO₄ and Freedom rootstocks on 8 g/L PEG medium had shortened their roots (1.88 and 1.86cm).

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