



Original Research Article

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In Vitro Radiosensitivity for Two Genotypes of Potato (*Solanum tuberosum* L.) and Physiological Studies under Salinity

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Abstract

In order to determine the optimal radiation dose, the sensitivity of Riviera and Burren genotypes to gamma rays was tested. Cuttings exposed to gamma irradiation of 0, 5, 10, 15, 20, 25 and 30 Gy and placed in callus induction medium, which showed that 0, 5, 10 and 15 Gy gave higher percentage reached 100% with shorter times (10-12 days) for each genotypes while 20 and 25 Gy caused reduction in callus induction and increasing the time taken for induction. Results based on callus growth criteria showed the optimal doses for mutation were 12 and 18 Gy for Burren and Riviera respectively. Irradiated and non irradiated calli were planted in medium supplemented with different salt levels (6, 8, 10, 12 dS m⁻¹). At all salt levels, radiation decreased callus fresh weight, relative growth rate and water content and increased accumulation of K⁺, Na⁺, Cl⁻ and proline. Ca⁺⁺ and carbohydrate accumulations was not affected by radiation. In both genotypes, salinity stress decreased callus fresh weight, relative growth rate and water content and increased Na⁺, Cl⁻ and proline accumulations.

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Introduction

Radiosensitivity has been defined as a measure of cells susceptibility when exposed to radiation. Biological effects of Radiation are determined by directed and in direct, The first one caused damage in genetic material (DNA) especially if absorbed energy was enough to root out electrons from DNA molecule leading to break occurs for one or both of strands causing a change in the genetic code or leading to cell death. While the second, caused decomposition of water and be free radicals (Kovac and Keresztes, 2002).

breeding program by induction and mutant breeding (FAO/IAEA, 1977; Ibrahim et al., 1990; Van Hartin, 1998). On this side, many studies mentioned to these sensitivity for many varieties of Potato growing *in vitro* (Sonnino et al., 1986; Hassan and Javed, 1991; ALSalhi, 2002). Salinity is a serious problem, affect plant growth and productivity in many crop. Many parameters can be used to determine tolerance to salinity stress such as fresh and dry weight, ion, proline and carbohydrates accumulation. Potato has been classified as a moderately salt sensitive with thresholds 1.7 dS m⁻¹ (Maas and Hoffman, 1977).

Radiosensitivity test is a necessary priorities in plant

Yaycili and Alikamanoglu, 2012 studied many

parameters (percentage of regeneration, plant height, number of leaves and the percentage of roots formation) after exposed stem cuttings of Marfona cultivar to different doses of Gamma rays, They found that 18 Gy caused reduction 30% in all parameters except plant height, while 26, 23 and 20 Gy caused reduction 50% in percentage of regeneration and number of leaves, root formation and plant height, respectively. Also AL-Safadi et al. (2000) found that 2.5 Gy caused an increase number of microtuber (38%) while the weight of microtuber was not affected and they concluded that this dose possible use in promoting microtuber formation without caused variation.

The objective of this study is expand of genetic variation by using radiation (Gamma rays) and determining the optimal dose which caused reduction 50% in fresh weight and using physiological markers as an indicator to salt tolerance for two genotypes of Potato (Riviera and Burren).

Materials and methods

The research was conducted at Genetic Engineering Department in Agricultural Research Directorate in Ministry of Science and Technology/Iraq. Potato varieties (Riviera and Burren) were micropropagated on MS (Murashige and Skoog, 1962) nutrient medium; pH was adjusted to 5.7 prior to autoclaving at 121°C for 20 minutes.

In vitro radiosensitivity

For radiosensitivity, Potato plantlets of Riviera and Burren were exposing with different doses (5,10 ,15, 20 and 25 Gy) of Gamma rays (source Co ⁶⁰ with activity 243 Gy hour⁻¹). intermodal segments (1-1.5 cm) were excised and placed on Petri dishes containing callus induction medium MS with 3% sucrose, 8% agar and 0.1, 100, 0.5, 0.5, 2, 2 mg L⁻¹ of Thiamine –HCL, Inositol, Glycin, Nicotinic Acid, BA and 2,4-D respectively. The cultures were incubated in growth room chamber at 25°C±2 under 16 h light and 8 h dark. Data of % callus induction, number of days required for callus induction, callus morphology, callus fresh weight (mg) were taken. The optimal dose was calculated as 50% reduction in fresh weight of callus as follows:

% Reduction = $\left(\frac{\text{Callus fresh weight in control treatment} - \text{Callus fresh weight in irradiated treatment}}{\text{Callus fresh weight in control treatment}} \right) \times 100$

In vitro salt tolerance

After determined the optimal dose, 12 and 18 Gry for Burren and Riviera genotypes respectively, stem segments (approximately 1- 1.5 cm size, without node) were excised from irradiated and non irradiated *in vitro* plantlets and planted in the previous medium supplemented with different levels of NaCl to generate EC at 8, 10, 12 dSm⁻¹, the EC of the control treatment (MS basal medium, without adding NaCl) was 6 dSm⁻¹. All cultures were placed in a growth room chamber under the same light and environmental conditions as previously stated. After 30 days several characteristics were recorded. These include:

Callus fresh weight (mg): callus was divided into pieces of 150 mg (initial fresh weight), 5 pieces were placed in Petri dishes containing MS medium supplemented with different levels of EC (6, 8, 10 and 12 dSm⁻¹) after one month final callus fresh weight were recorded.

Relative Growth Rate (RGR) was calculated following the formulae of Lutts et al. (1998): $RGR (mg \times 10^{-2} gm^{-1} \text{ callus fresh weight/day}) = \frac{\ln W_2 - \ln W_1}{\Delta t}$ Where W1 refers to initial fresh weight. W2 refers to final fresh weight. Δt refers to the time for culturing (30 day).

Water content estimated according to Forooghian and Esfarayeni (2013) Relative Water Content of callus = $\frac{(\text{Wet weight of callus} - \text{Dry weight of callus})}{\text{Wet weight of callus}} \times 100$ Callus dry weight was determined after 2 days oven dried at 60°C.

Determination of ions content: 150 mg dry weight callus was placed in beaker containing 9 ml digesting mixture (10 Nitric acid: 4 Perchloric acids: 1 sulfuric acid). The beakers were heated up to 60 °C until the solution became colorless then the digestion diluted with distilled water. Concentrations of Ca⁺⁺, Na⁺ and K⁺ were measured using Atomic Absorption Spectrophotometer (Shimadzo AA-670) according to the manufacturer's recommendation, While Cl⁻ estimated by digesting with 37.5 mg Cao and 90 ml distilled water in ceramic container. The container placed on sand path until the solution evaporation then transferred to furnace oven at 550°C for 2 hrs. Sample was cool and added hot distilled water and filtered. completed volume (100 ml) with 5 drops of potassium chromate (1%) . 10 ml from solution placed on beaker and flow with Ag No₃ (0.05 N) until the solution became pink. Concentration of Cl⁻ was measured using following the equation:

Mg^{-1} Cl callus dry weight = $[\text{Ag No}_3(\text{ml}) \times \text{Ag No}_3(\text{N}) / \text{Sample volume}] / \text{dilution factor}$.

Determination of carbohydrate content: 200 mg fresh weight callus was placed in test tube containing 1 ml distilled water then digested and centrifuged at 1500 rpm min^{-1} for 10 minute. 20 micro liter from phenol indicator (5% weight /volume) was added to 300 micro liter of the sample then adding 150 micro liter H_2SO_4 . Sample was incubated in path water at 25-35°C for 20 min. colored intensity was determined by measured optical density using microplate reader spectrophotometer at wavelength 488 nanometer. Concentrations of carbohydrate was measured as follow as: $\text{mg glucose gm}^{-1}$ callus fresh weight = (reading device / dilution factor) \times the volume of reading.

Determination of proline content: 30 mg dry weight callus was taken and mixed with 800 micro liter sulphalicylic acid (3%) then digested and centrifuged at 2000 rpm min^{-1} for 10 minute. 0.5 ml from acetic acid and 0.5 ml ninhydrin solution adding to mixture solution (0.5 ml) then incubated in path water for 30 min. Red layer isolated by adding 2 ml toluene then colored intensity was determined by measured optical density using microplate reader spectrophotometer at wavelength 520 nanometer. Concentrations of proline was measured as follow as:

$\text{Micromole proline gm}^{-1}$ callus dry weight = $[(\text{microgram proline/ml} \times \text{ml toluene}) / 115.5 / \text{micro mol}] / \text{dilution factor}$.

Results were statistically analyzed using GenStat program and means were separated using Duncan's test at a probability level of 5%.

Results and discussion

In vitro radiosensitivity

Results in Table 1 revealed that in both cultivars, % callus induction significantly increased with increasing radiation doses at 0, 5, 10 and 15 Gy with days required for callus induction ranged 10-12 days. While the doses 20 and 25 Gy caused significantly decreased in callus induction (52.0 and 44.0% , 68.0 and 64.0% for Riviera and Burren respectively), with increasing number of days required for callus induction ranged 12-14 days. The results in Table 2 showed that callus fresh weight significantly decreased with increasing irradiation doses in both genotypes. Callus growth was different among genotypes in the control treatment (non irradiated) and irradiated at 5 Gy, the highest callus fresh weight was recorded in Burren (548.00 and 566.42 mg) respectively. Callus color in the control treatment ranged from green - compact (Burren) to greenish compact with white area, while the color at 15, 20 and 25 Gy ranged from green - compact with brown to greenish brown compact for both genotypes (Table 2; Figs. 1 and 2). Decreased in all callus characterize with increasing radiation doses may be due to decrease in internal growth regulators, especially cytokines, as a result of their fragmentation or lack of manufacturing (Omar et al., 1993).

Table 1. Effect of gamma doses on % callus induction, number of days required for callus induction, fresh weight and callus morphology of potato genotypes.

Gamma doses Gy	% callus induction		Number of days required for callus induction		Fresh weight (mg)		Callus morphology (color-texture)	
	Riviera	Burren	Riviera	Burren	Riviera	Burren	Riviera	Burren
0	100a	100a	10.00e	10.80d	404.00b	548.00a	greenish compact with white area	green - compact
5	100a	100a	11.00d	11.00d	398.92b	566.42a	greenish	greenish with purple area, compact
10	100a	100a	11.00d	11.00d	289.04bcd	295.72bc	green- compact with roots	Compact – with green area and purple area
15	100a	100a	12.00c	12.00c	250.00cd	252.00cd	green- compact with brown	Compact – with green area and purple area
20	52.0b	44.0b	12.70b	12.80b	172.00cde	54.00e	green- compact with brown	Compact – with green area and brown area
25	68.0b	64.0b	13.90a	14.00a	144.00de	68.00e	green- compact with brown	greenish brown compact

The average which have the same letter not significant difference according to Duncan polynomial test at 5% level.

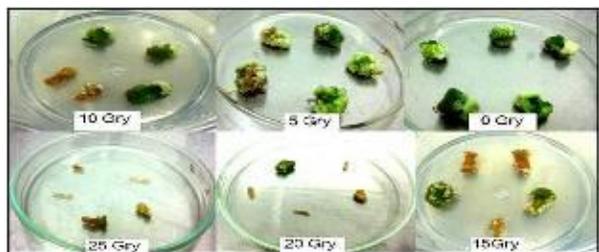


Fig. 1: effect of gamma rays on callus phenotypes for Burren genotype.

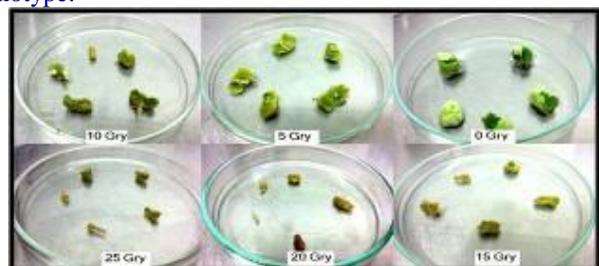


Fig. 2: Effect of gamma rays on callus phenotypes for Riviera genotype.

Determination of the optimal dose

Results in Fig. 3 showed that the percentage of reduction in fresh weight of callus which induced from Riviera and Burren genotypes increased with increasing irradiation doses. The optimal dose, which gave 50%

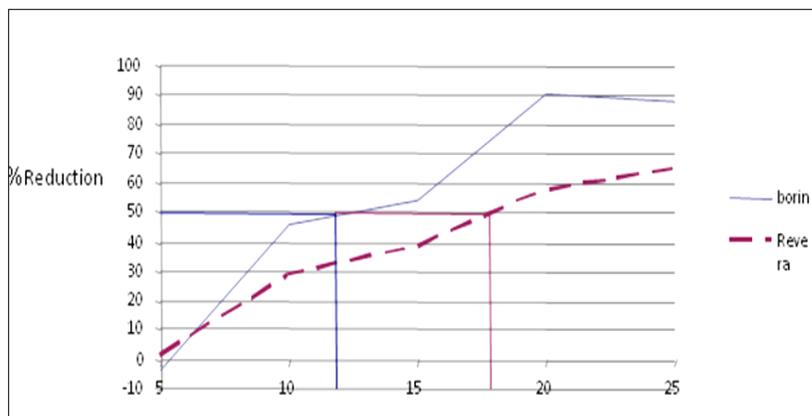


Fig. 3: Effect of different doses of Gamma rays on callus fresh weight for Riviera and Burren genotypes.

The result for Relative Water Content of callus is given in Fig. 2 showed that irradiation caused significant decrease in the Relative Water Content at 12 dS m⁻¹ in Riviera tissue (88.09) compared with other levels, while non irradiated treatment caused the same effect at 12 dS m⁻¹ for both Riviera and Burren genotypes (83.80 and 85.60). It is clear from the results that salinity has caused reduction in fresh weight, Relative growth Rate and Relative water content may be related to the tissue through the total energy availability of stress metabolic

reduction in the fresh weight, was 12 and 18 Gy for Burren and Riviera respectively. By dotting points, and these two doses were adopted to cause variations in subsequent experiments. The reduction in growth of 30-50% is a criterion for the optimal dose at which a sufficient quantity of plants grows later and produces viable seeds that produce mutant plants that are non sterile (AL-Takretti, 2002; van Harten, 1998).

Effect of salinity and irradiation on physiological indicators

Results in the Table 2 showed that in non irradiation treatment significant superiority of the fresh weight of Riviera genotype compared with the Burren genotype in all salt levels (1542.6, 2098, 1028.3 and 1348.1 mg at 6, 8, 10 and 12 dS m⁻¹ Respectively), while in irradiation treatment there was no significant difference in both genotypes.

As for the Relative Growth Rate (RGW), irradiation has a significant effect on the growth of Burren genotype at salt levels 8 and 10 dS m⁻¹ (1.84 and 1.63 gm gm⁻¹ callus fresh weight x10⁻² respectively), while it not significantly affect on Riviera genotype under all salt levels compared with non irradiation treatment (Table 2).

processes, which delays callus growth (Cushman et al., 1990), or readiness of water and soluble nutrients in the salt medium, which is reflected on cell division and growth.

Also Queiros et al. (2007) explained the reduction in the water content may be due to the high osmotic pressure of the culture medium with high salt concentration. The inhibitory effect of irradiation, either as a single factor or in combination with different salinity levels, has been

noted by several studies (Aldemita and Zapata, 1991; AL-Tikriti, 2002) due to increased radiation damage at the

level of chromosomes in the merismatic cells and delay the division process (Yaycili and Alikamanoglu, 2012).

Table 2. Effect of radiation and salt levels on Fresh weight, Relative growth rate and Relative water content for two genotypes (Riviera and Burren) after 30 days.

Radiation	Genotypes	Salt levels dS m ⁻¹			
		6	8	10	12
Fresh weight (mg)					
Non-irradiation	Riviera	2098.6a	1542.6b	1348.1bc	1028.3cd
	Burren	506.3e	475.1e	442.1e	266.3e
Irradiation	Riviera	748.73de	520.87e	397.30e	317.83e
	Burren	414.97e	345.4e	306.43e	280.67e
Relative growth rate (gm gm⁻¹ callus fresh weight x10⁻²)					
Non-irradiation	Riviera	4.22ab	3.40abc	2.55cdef	2.05defg
	Burren	3.11bcd	4.19ab	3.06bcd	2.88cde
Irradiation	Riviera	4.35a	2.71cde	1.38fg	1.144g
	Burren	2.68cde	1.84defg	1.63efg	1.65efg
Relative water content (%)					
Irradiation	Riviera	90.66ab	91.47a	90.80a	88.09cde
	Burren	90.10abc	90.29abc	90.21abc	90.38abc
Non-irradiation	Riviera	90.35abc	89.48abcd	87.03ef	83.80g
	Burren	89.89abc	88.18bcde	87.29def	85.60fg

Means followed by the same letters are not significantly different (P<0.05) according to Duncan's test.

The results in Table 3 showed that Na⁺ increased significantly with increasing salinity in both irradiated and non irradiated treatments. Riviera and Burren genotype accumulated higher Na⁺ at salt levels 10 and 12 compared with 6 and 8 dS m⁻¹ in irradiated treatments reached 40.00, 43.75 and 35.00, 45.00 gm gm⁻¹ callus dry weight respectively. There was no significant effect of irradiation on Cl⁻ accumulation at some salt levels in both genotypes (Table 3).

On the other hand, there was a significant difference between two genotypes at salt levels 8, 10 and 12 dS m⁻¹ in non irradiation treatment and at 10 and 12 dS m⁻¹ in the irradiation treatment, Also showed that Burren accumulated Cl⁻ higher than Riviera genotype at 12 dS m⁻¹ in both non and irradiated treatments (63.00 and 60.00 respectively).

The result presented in Table 4 shows that in both genotypes tissue irradiation significant decrease K⁺ accumulation at salt levels 8,10, 12 dS m⁻¹ compared with non irradiated treatment (5.20 , 15.10 , 11.10 gm gm⁻¹ callus dry weight and 5.20 , 3.05, 4.60 gm gm⁻¹ callus dry weight for Riviera and Burren respectively). It appears from the results in Table 4 that there was no significant difference in Ca⁺⁺ accumulation at all salt levels in both genotypes in non irradiation except at level 8 dS m⁻¹. In irradiation treatment, Ca⁺⁺

accumulation significantly decreased at salt levels 8 and 10 dS m⁻¹ (4.30 and 4.20 gm gm⁻¹ callus dry weight respectively) compared with 6 and 12 dS m⁻¹ (6.50 and 7.35 gm gm⁻¹ callus dry weight respectively) in Riviera genotype while, in Burren genotype significantly reduced Ca⁺⁺ at 12 dS m⁻¹ (4.60 gm gm⁻¹ callus dry weight) compared with level 8 dS m⁻¹ (6.75 gm gm⁻¹ callus dry weight). It seems that both genotypes significantly different in their accumulation of Na⁺, Cl⁻, K⁺ and Ca⁺⁺, may be due to genotype dependent which are reflected in their ability to take and transfer ions within tissue. Salinity was significantly affected by accumulation of ions, which showed that Ca⁺⁺ was Oscillating with increasing accumulation of Na⁺ and Cl⁻ by increasing salt levels, which were associated with decrease in K⁺, especially at 12 dS m⁻¹. This may be explained by the cells in the callus tissue are working to allow these ions to enter to increase osmotic pressure causing accumulation of ions in cytoplasm and vacuole as a result of salt stress. These results were agreed with Munns (1993), AL-Dahimawi (2009) and Shojaie (2010). While irradiation significantly affected by increasing Na⁺ with decreasing K⁺ this may be related to affect on mechanism of ion absorption by damage to the cellular membranes and thus affect membrane permeability or ion transport system. These results were consistent with the results of Paschinger and Vanicek (1974) and Mulawee (1980).

Table 3. Effect of radiation and salt levels on sodium Na⁺ and chloride Cl⁻ ions (gm gm⁻¹ callus dry weight) for two genotypes (Riviera and Burren) after 30 days.

Radiation	Genotypes	Salt levels dS m ⁻¹			
		6	8	10	12
Na⁺ (gm gm⁻¹ callus dry weight)					
Irradiation Non	Riviera	15.32ef	21.53de	26.35d	25.27d
	Burren	12.91f	25.15d	24.88d	25.08d
Irradiation	Riviera	22.50de	26.25cd	40.00ab	43.75a
	Burren	13.65f	33.75bc	35.00b	45.00a
Cl⁻ (gm gm⁻¹ callus dry weight)					
Non-irradiation	Riviera	10.50g	14.90efg	28.00def	43.65bc
	Burren	14.00fg	31.50cd	52.75ab	63.00a
Irradiation	Riviera	11.40g	26.40def	29.50cde	30.65cd
	Burren	16.65defg	26.25def	48.90ab	60.25a

The average which have the same letter not significant difference according to Duncan polynomial test at 5% level.

Table 4. Effect of radiation and salt levels on potassium (K⁺) and calcium (Ca⁺⁺) ions (gm gm⁻¹ callus dry weight) for two genotypes (Riviera and Burren) after 30 days.

Radiation	Genotypes	Salt levels dS m ⁻¹			
		6	8	10	12
K⁺ (gm gm⁻¹ callus dry weight)					
Non-irradiation	Riviera	21.20b	27.97a	21.73b	10.61cdefg
	Burren	14.16cd	12.02cde	10.88cdefg	7.66efgh
Irradiation	Riviera	8.40defgh	5.20fgh	15.10c	11.10cdef
	Burren	5.45fgh	5.20fgh	3.05h	4.60h
Ca⁺⁺ (gm gm⁻¹ callus dry weight)					
Non-irradiation	Riviera	5.69abcdef	4.37def	3.69f	6.07abcde
	Burren	6.60abc	6.90a	5.93bcdef	6.32abcd
Irradiation	Riviera	6.50abc	4.30def	4.20ef	7.35a
	Burren	6.05abcde	6.75ab	4.70bcdef	4.60cdef

The average which have the same letter not significant difference according to Duncan polynomial test at 5% level.

Data related to Proline accumulation is given in Table 5. Results showed that irradiation led to reduction in Proline accumulation in Riviera genotype at salt levels 8 and 10 dS m⁻¹, whereas otherwise in salt levels 6 and 12 dS m⁻¹. While in Burren genotype noted that irradiation increased proline accumulation at all salt levels compared with non irradiation. Many authors mentioned that the important of Proline in salt tolerance as a role in a protective agent for enzymes (Solomon et al., 1994) and cell organelles in cytoplasm (Van Rensburg et al., 1993). As well as, considered a carbon-nitrogen and free-radical compound of free radicals scavenger (Chinnusamy et al., 2005). Increased accumulation of proline in cells is a common response in plants under salt stress (Szabados and Savouré, 2010; AL-Shammari, 2001 and AL-Tikriti, 2002) as a results of its high rates of manufacture and low oxidation (Kumar et al., 2003). Results in Table 5 showed that significant decrease in carbohydrate accumulation in callus of Burren genotype at salt level 8 dS m⁻¹ (2.14 mg glucose g⁻¹ callus fresh weight) compared with 6 dS m⁻¹ (4.19 mg glucose g⁻¹ callus fresh weight) in non irradiated

treatment. On the other hand, irradiation had no significant effect on carbohydrates accumulation in both genotypes at all salt levels exception of salt level 6 dS m⁻¹ in Burren genotype where carbohydrate decreased (4.19 and 1.92 mg glucose g⁻¹ callus fresh weight in non- irradiated and irradiated treatment respectively).

The decrease in the carbohydrate content under some salt levels was consistent with results of AL-Shammari (2001), which found a significant decrease in carbohydrates in callus of some varieties of sugarcane reached 16 - 47% as compared with control treatment, explaining that in spite of salinity caused Stimulate carbohydrate metabolism enzymes, salt stress at the same time has led the cell to release a large portion of energy, to resist ionic disturbance within the cell, or may be due to transform of carbohydrates to starch by increasing salt stress (Stavarek and Rains, 1985; Omar et al., 1993). The negative effect of radiation on carbohydrates accumulation in some salt levels was agreed with results of Kebeish et al. (2015).

Table 5. Effect of radiation and salt levels on carbohydrate and proline (gm gm⁻¹ callus dry weight) for two genotypes (Riviera and Burren) after 30 days.

Radiation	Genotypes	Salt levels dS m ⁻¹			
		6	8	10	12
Carbohydrate (mg glucose gm⁻¹ callus fresh weight)					
Non-irradiation	Riviera	2.86ab	2.59ab	2.64ab	2.99ab
	Burren	4.19a	2.14b	3.13ab	2.30b
Irradiation	Riviera	2.34b	2.99ab	3.29ab	2.06b
	Burren	1.92b	3.20ab	2.99ab	3.27ab
Proline micromole proline gm⁻¹ callus dry weight					
Non-irradiation	Riviera	10.89cde	37.65a	23.55bc	6.20e
	Burren	8.89de	12.95cde	6.66e	11.47cde
Irradiation	Riviera	16.63cde	14.13cde	12.48cde	23.90abc
	Burren	22.24bcd	30.53ab	18.94bcde	13.29cde

The average which have the same letter not significant difference according to Duncan polynomial test at 5% level.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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