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Effect of Gamma Radiation on G1 Rhizome Yield and Germination of *In Vitro* Tissue Cultured Jamaica Yellow and Blue Ginger (*Zingiber officinale Roscoe*)

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ABSTRACT

Data was analyzed from G1 rhizomes harvested April 2025 from ginger (Zingiber officinale Roscoe var. Jamaica blue) at the Biotechnology Department of the Jamaica Scientific Research Council. Tissue culture (TC) explants were irradiated at the Plant Breeding Unit, Joint FAO/IAEA Agriculture and Biotechnology Laboratory, IAEA Laboratories, Siebersdorf, Austria in 2018. The radiation source was 60Co - 4.30 Gy/min. Based on LD50 results of the radio-sensitivity studies, bulk gamma irradiation was conducted on Jamaica blue ginger TC explants using doses 8, 12 and 16Gy. The majority of the observations were on 12Gy lines (53%) and 16Gy lines (37%). Harvested rhizomes bore anomalies including considerable roots, some linear, slender, swollen and with terminal nodules. Most lines bore 1 - 3 rhizomes (53%), others 4 - 6 rhizomes (37%) and up to 10 and 13 with low frequency. The mean rhizome mass ranged from 6.5 ± 5.0 g to 20.0 ± 5.0g per line. The mean mass of roots ranged from 1.4 ± 1.3 to 13.59 ±2.31g, observed for line 12Gy50. This mean mass was significantly higher than the other lines. Germination or sprouting of the rhizomes of irradiated lines ranged from 75 to 91% compared to the control 60%. Sprouting was significantly higher for 12 Gy lines than the control with t = 4.26, for 12 df, P = 0.05. The mean shoot length for irradiated lines was significantly lower than the control. The mean shoot lengths were 15.99 ± 4.18 cm, 15.77 ± 7.1 cm and 26.28 ± 3.24 cm for 12Gy, 16Gy and control respectively. Four mutant lines with significantly lower shoot lengths were identified 12Gy102, 12Gy114, 16Gy23 and 16Gy26.

Keywords

Gamma radiation, Uield, mutations, G_1 ginger rhizomes, Tissue culture, Jamaica ginger, Shade-house production, protective cover, Pot culture.

Introduction

The Biotechnology department of the Jamaica Scientific Research Council (SRC) has a long history of research in ginger mutation breeding. Reports cite as early as 2007 [1,2], while 2012 was also reported as a significant time line for SRC's collaboration with the International Atomic energy Agency (IAEA) in Vienna [3]. The IAEA has supported research worldwide in a number of crops, but its first support for nuclear energy in ginger improvement anywhere in the world, was to Jamaica SRC [4]. The Jamaica SRC has become a leader in the Anglophone Caribbean with respect to mutation breeding by Gamma radiation.

Breeding for disease resistance in ginger is a lengthy process requiring years of field trials and testing before improved varieties are released commercially. Historically, the evaluation of tissue culture ginger plants required at least two to three crop seasons to realize normal size rhizomes [5,6]. Conventional breeding also

takes at least 10 to 12 years [4]. This current work was started 2018. It involved several years of bulking irradiated lines *in vitro*, preliminary *in vitro* screening, continued bulking, then weaning and hardening. The procedure followed prescribed protocols for producing G_1 rhizomes in the shade-house, harvest of rhizomes, and application of single bud technology (SBT) to generate sprouted buds for field planting [7].

Gamma radiation breeding is a relatively random approach to breeding and is not so targeted like current advanced CRISPR and gene editing methods [8,9]. Other non-target or bystander traits may be affected in the irradiation process. There are widely varying reports on the effects of gamma radiation treatment for ginger improvement. Some indicate no effects in constituent bioactives [10] while some report changes [11]. Radiation up to 10 Gy is reported to cause insignificant morphological or phenotypic changes in plant chemicals [12]. Different doses of mutagen cause different effects on plants and it is highly variety-dependent [13]. The spectrum of mutant characters commonly spans stunted growth, plant stature, leaf deformation, and chlorophyll mutation.

At SRC, one of the research interests is induction of resistance in ginger to the rhizome rot complex. This complex describes the rots caused by *Phytium* spp., leaf yellowing arising from *Fusarium* spp. and bacterial wilt associated with *Ralstonia solanacearum* among other pathologies [14-16]. If radiation is applied to successfully achieve resistant mutants, concerns may arise about possible effects of Gamma rays on sprouting. Yusof [17] showed that irradiation inhibited sprouting in ginger when compared to the non-irradiated samples. It is also felt that gamma radiation itself may affect ginger lines with variable responses between lines as observed by variable *in vitro* multiplication rates, selection for survivors at weaning, differences in vigor and growth between lines.

This segment of the research is part of a larger activity involving FAO/IAEA funded research to deliver gamma radiated mutant lines that are resistant to ginger rhizome rot. This investigation was motivated by unsubstantiated and anecdotal observation of variable multiplication rates between radiated lines, low shade-house survival and rhizome yield. The purpose of the research is to investigate possible effects of Gamma radiation (8, 12 and 16 Gy) on rhizome yield of tissue culture ginger studying G_1 rhizomes harvested at random from pots maintained under protective cover in the shade-house.

Methodology

Summer 2018, Jamaica blue and Jamaica yellow ginger tissue culture (TC) explants were irradiated at the Plant Breeding Unit, Joint FAO/IAEA Agriculture and Biotechnology Laboratory, IAEA Laboratories, Siebersdorf, Austria. The radiation source was ^{60}Co - 4.30 Gy/min. Based on LD_{50} results of the radio-sensitivity studies, bulk gamma irradiation was conducted on Jamaica blue ginger TC explants using doses 8, 12 and 16Gy. The Jamaica blue explants were returned to SRC Biotechnology Department for *in vitro* multiplication of 200 lines. Preliminary screening using adapted detached leaf assays [18-20] and resistance evaluation by conductivity bioassay [21,22] identified 21 lines with putative resistance to rhizome rot.

These ginger lines were weaned and grown in soilless media in pots under protected cover leading up to 2023 - 2024. April 2025 G_1 rhizomes were harvested in preparation for field trial to evaluate disease resistance. Rhizome numbers and rhizome yield was recorded for 19 available lines. After one week's storage, 4°C, rhizomes were cut into mini setts for sprouting by single bud technology (SBT) and germination rates recorded. The number of emerged shoots and plant height was recorded at 60 days.

Frequency distribution plots were prepared to show the frequency of lines in different radiation treatments, as well as the frequency of ginger lines producing multiple rhizomes. Comparison of means of several samples was computed using pair-wise application of the *t*–test where ANOVA was not applicable:

$$t = \frac{\bar{x} - \bar{x}}{\sqrt{\left[\frac{(n-1)s^2 + (n-1)s^2}{n+n-2}\right]\cdot\left[\frac{n+n}{n\,n}\right]}}$$

ANOVA using log *x* transformed data determined differences between means of count data. Where ANOVA F value was significant, the Tukey test statistic T was computed to identify samples that were significantly different [23].

Observation and results

The nineteen reputed resistant lines available for rhizome harvest were not equally distributed among dosage treatments with respect to numbers or equal representation. The skewed nature of the line selection meant that replication across treatment groups was no applicable. There were few lines in 8Gy. A summary of relative distribution of lines among treatment classes showed that half (53%) of the lines were irradiated at 12Gy. Most of the remaining lines (37%) were treated at the higher dosage of 16Gy. Only 20% of the resistant lines resulted from the lowest 8Gy treatment (Figure 1).

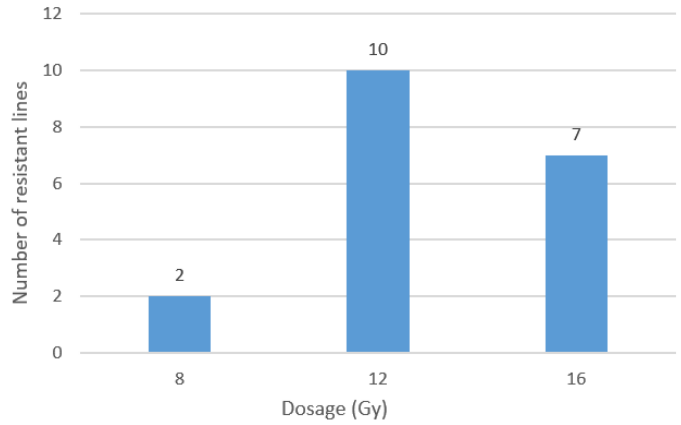

Figure 1: Distribution of SRC resistant lines among three radiation dosages (Gy).

The harvested G_1 rhizomes showed unusual morphologically features besides small size that set them apart. The linear shape, the different types of roots attached to some rhizomes, and swollen ends or nodules on these swollen roots were interesting. The rhizomes of irradiated ginger lines tended to be slender and linear, with segmented growth indicative of the sequential production of new rhizome tissue along a chain in a one-dimensional plane (Figure 2). What was interesting was the constrictions at regular intervals each indicating a possible single bud for continued propagation.

In additional to a noticeable reduction in the variety of shapes, predominantly linear, there were other interesting shapes. Figure 3 shows linear growth of the rhizome outwards in several directions from a central point. The new growth and old growth was differentiated by conspicuous color change along the rhizome. New segments were formed in a restricted axis. Active growth was shown at all the terminal ends simultaneously, but growth was directed in the same axis or dimensional plane.

Figure 2: Slender, linear rhizomes.

Figure 3: Irregular shaped rhizomes.

Not all rhizomes bore attached roots at harvest. Rhizomes appeared to be at varying stages of morphological development. Where roots were present, some were thin and threadlike, while others were fat and succulent (Figure 4). There were mutant ginger lines which appeared to produce as much swollen root as rhizome biomass. One feature of the swollen roots was further swelling and differentiation into a round structure or nodule at the terminal end (Figure 5).

Figure 4: Swollen roots harvested with rhizomes.

Table 2 summarized the mean number of rhizomes between ginger lines treated with different radiation doses and provided data for the *t*–test. The pairwise application of the *t*-test indicated that there was no difference in the mean number of harvested rhizomes between lines of the three dosage treatments. The calculated *t* values 0.435,

0.048 and 0.839 for pairwise comparisons 8Gy:12Gy, 8Gy:16Gy and 12Gy:16Gy respectively did not exceed the tabled critical value of 2.23, 2.365 and 2.13 for 10, 7 and 15 *df* respectively (P = 0 .05). In the Figure 6 illustration 12Gy has the highest mean number of rhizomes per plant but with the wide variance this is not significantly different from the other means (P = 0.05).

Figure 5: Terminal nodule on rhizome roots.

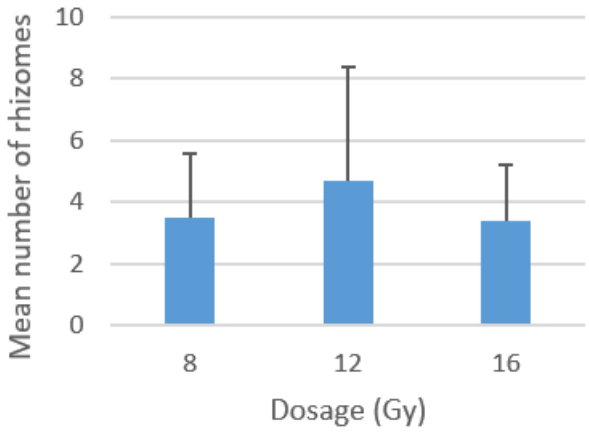

Figure 6: Distribution of number of rhizomes among dosage treatments.

Table 2: Summary of rhizome numbers between lines of different Gamma radiation doses (Gy).

	8Gy	12Gy	16Gy
n	2	10	7
$\bar{x}$	3.50	4.70	3.43
s	2.12	3.68	1.81
s^2	4.5	13.57	3.29

Figure 7: Frequency distribution of number of rhizomes among lines.

The data was pooled since there was no significant difference in rhizome numbers between dosage treatments. A frequency distribution plot of the number of rhizomes produced per ginger line showed that 53% of the ginger lines produced 1 - 3 rhizomes. Another 32 % produced between 4 - 6 rhizomes. Production of larger numbers of rhizomes was reduced, about 10%. Production of more than a dozen rhizomes by ginger lines was a rare occurrence (5%) (Figure 7).

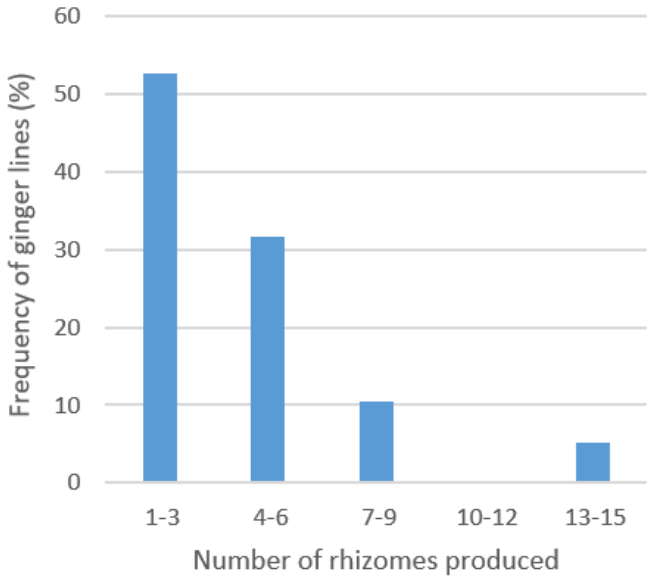

Figure 7: Frequency distribution of number of rhizomes among lines.

While 19 lines were harvested it was only possible conduct one way ANOVA on a smaller sample of 11 lines because of the low availability of replicated observations. The means and St. dev. of the mass of rhizome for each line are summarized (Table 3). With a wide range from 6 g to 20 g these mean rhizomes mass per line also had wide standard deviation ranges of 3 g to 11 g. In some cases the standard deviation was higher than the mean mass of the irradiated lines.

In the one-way ANOVA, the calculated value of F at 10 and 22 *df* of 1.89 did not exceed the tabled value of 2.30. As such, the difference in mean rhizome mass of the 11 samples where n = 3 in each case was not statistically significant ($F_{10,22} = 1.89$, $P < 0.05$) (Table 4). It was not possible to differentiate between irradiated lines using G_1 rhizome yield.

Table 3: Mass (g) of three largest rhizomes for irradiated ginger lines (mean ± St. dev).

	IAEA irradiated line numbers										
	5	**8**	**11**	**13**	**14**	**15**	**23**	**26**	**27**	**50**	**114**
	11.09	9.92	26.2	28.97	14.72	14.47	14.2	10.68	30.48	22.79	13.0 7
	7.62	7.96	16.45	15.1	10.31	5.08	5.38	4.73	13.46	14.81	10.78
	5.04	5.34	6.92	7.11	9.15	3.58	2.84	1.55	10.61	23.65	9.82
Mean	7.92	7.74	16.52	17.06	11.39	7.71	7.47	5.65	18.18	20.41	11.22
St. dev.	3.04	2.30	9.64	11.06	2.94	5.90	5.96	4.634	10.74	4.87	1.67

Table 4: ANOVA summary table for difference in rhizome mass between lines

Source of variation	SS	*df*	s^2	F
Between lines	810.180	10	81.018	1.89
Within lines	944.524	22	42.932	
Total	1754.704	32		

There were nine ginger lines which provided triplicate root mass data to conduct one way ANOVA, but due to lack of homogeneity of variances among them to satisfy the ANOVA F_{max} test, seven lines were compared. Table 5 summarized the means and St. dev. of the mass of roots for each line. The mean mass of roots per line ranged from 1.4 ± 1.3 g to 13.39 ± 2.31 g.

Table 5: Mass (g) of roots for irradiated ginger lines (mean ± St. dev.).

IAEA irradiated line number							
	5	**13**	**23**	**26**	**27**	**50**	**114**
	10.53	0.85	12.04	8.76	7.88	30.59	8.79
	7.05	2.32	3.89	3.25	10.12	15.22	4.57
	1.97	0.47	0.52	2.66	4.81	11.96	9.52
Mean	4.51	1.40	2.21	2.96	7.47	13.59	7.04
St. dev.	3.59	1.31	2.38	0.42	3.75	2.31	3.50

In the ANOVA the calculated F value at 6 and 7 *df* of 4.78 exceeded the tabled value 3.87. As such, the difference in mean root mass of the 7 samples where n = 3 in each case was statistically significant ($F_{6,7} = 4.78$, $P < 0.05$) (Table 6). The differences in root production are illustrated in Figure 8. The computed Tukey statistic T = 8.84 indicated that the mean root mass in line #50 was different from all other lines except line #27 and line #114.

Table 6: ANOVA summary table for difference in rhizome root mass between lines.

Source of variation	SS	*df*	s^2	F
Between	213.597	6	35.599	4.78*
Within	52.130	7	7.447	
Total	265.727	13		

When the rhizomes were sprouted for planting, the mean germination or emergence rates of the rhizomes in different treatment groups ranged from 60 to above 90% (Figure 9). The calculated *t* statistic for the pairwise comparison of mean germination between 12Gy and control lines was 4.26. This exceeded the tabled value of 2.18 at 12 df. The ginger lines irradiated at 12 Gy therefore had significantly higher germination than the control.

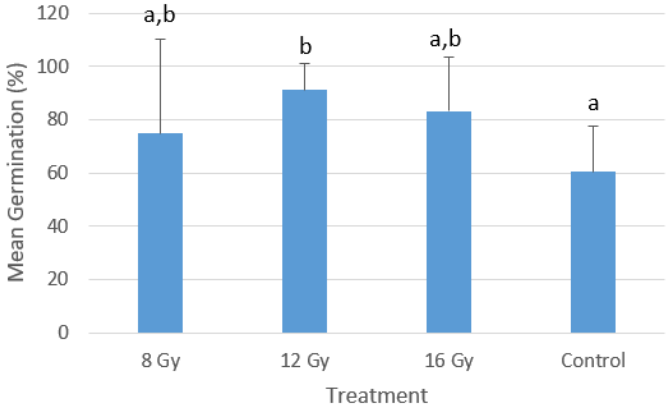

Figure 9: Mean germination rates of lines in different radiation treatment.

There was no difference in the number of sprouted shoots between radiation treatment groups (Table 6) but there was a difference with respect to shoot length. Pairwise application of the *t*-test showed that for 12Gy:control, $t = 4.97$ which exceeded the tabled critical value of 2.18 for 12 df, $P = 0.05$. For 16Gy:control, $t = 4.71$ which exceeded the tabled critical value of 2.62 for 9 *df*, $P = 0.05$. These mean shoot lengths for 12Gy and 16Gy were significantly different from control means (Figure 10).

Table 7: Summary of mean number of sprouted shoots produced by radiation treatment.

Treatment	Mean	St. dev.
8 Gy	6.5	3.5
12 Gy	11.6	4.5
16 Gy	7.3	3.6
Control	9.0	3.9

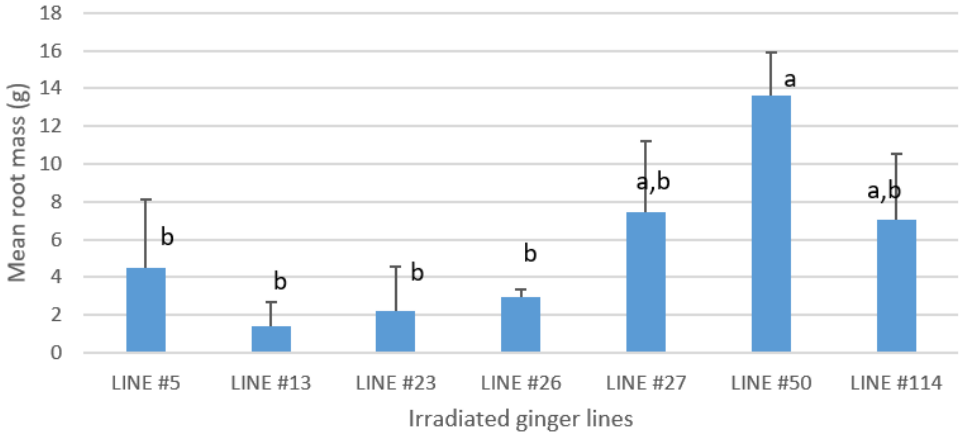

Figure 8: Difference in mean root mass (g) between ginger lines.

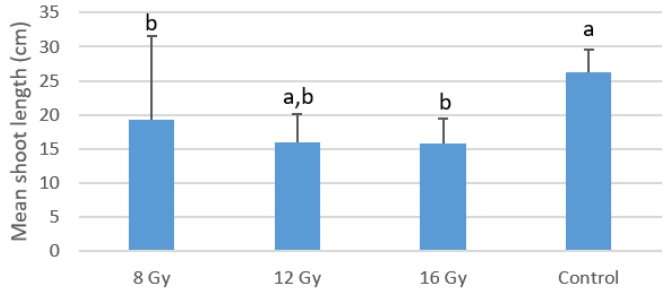

Figure 10: Difference in mean shoot length between radiation treatments.

In addition to differences in shoot length between radiation treatments, differences between lines were identified. ANOVA of mean shoot lengths for a random sample of 15 lines, a sample group that met the homogenous variance criteria, where n = 6 showed that the difference in mean shoot length was statistically significant ($F_{14,75} = 6.77$, $P < 0.05$). The ANOVA is summarized in Table 7. The critical tabled value was between 1.8 and 1.9.

Table 8: ANOVA summary table for difference in shoot length between lines.

Source of variation	SS	*df*	s^2	F
Between	2.637	14	0.188	6.766*
Within	2.089	75	0.028	
Total	4.727	89		

The Tukey statistic, T = 0.32 identified 18 out of a total of 105 pairwise comparisons that were significantly different. The most readily detectable significant differences included the shortest shoot length, 12 Gy102. This was significantly different from 12 Gy11, 12Gy27 and 12Gy50. The other short shoot lengths were 12 Gy114, 16Gy23 and 16Gy26. These were also significantly different from 12Gy27. These lines with short shoot length were also significantly different from the controls (Figure 11).

Discussion

Interesting benchmark data has been generated on G_1 rhizomes harvested under the specific shade-house or uncover protects conditions at Jamaica SRC. This provides a useful reference for future research work to update expand. This preliminary study alerts us to the need for expertise of rhizome or root crop specialist to resolve the morphological features of the rhizomes from the mutant ginger lines. The impact of radiation on yield of G_1 rhizomes and how this is translated to field grown ginger is speculative. What is more interesting from the research is the finding that irradiated lines showed significantly improved sprouting or germination than controls and secondly, sprouted shoots of irradiated lines showed significant reduced shoot lengths than the control.

The effects of Gamma radiation were earlier described as not targeted. So there is a possibility that induction of disease resistance may be simultaneously associated with a reduction in yield. Decreases in yield have been reported in some ginger varieties at 5 Gy [13,24]. The yield is expected to decrease with increasing dosage, but this is speculation and subject to confirmation by experimentation. It is however a positive result that there was no correlation between number or mass of rhizomes and the radiation dosage. It is nonetheless a desirable outcome and serendipitous if there is any advance on yield over the controls.

In the context of pot culture and shade-house or undercover protective maintenance, the mean rhizome mass from 6.5 ± 5.0 g to 20.0 ± 5.0 g appears significantly lower than published data. Fresh weight of rhizomes in sand has been reported at 40 - 50 g and higher depending on substrate [25]. The recommended weight of a single bud for propagation is 4 - 6 g so the observed SRC rhizome mass at least meets the criteria for continued propagation using SBT [7, 26,27]. The numbers of shoots derived from single buds indicated that we were able to germinate buds that were smaller than the prescribed 5 g. Varietal differences must so be appreciated. It is also noted that the SRC rhizomes were produced

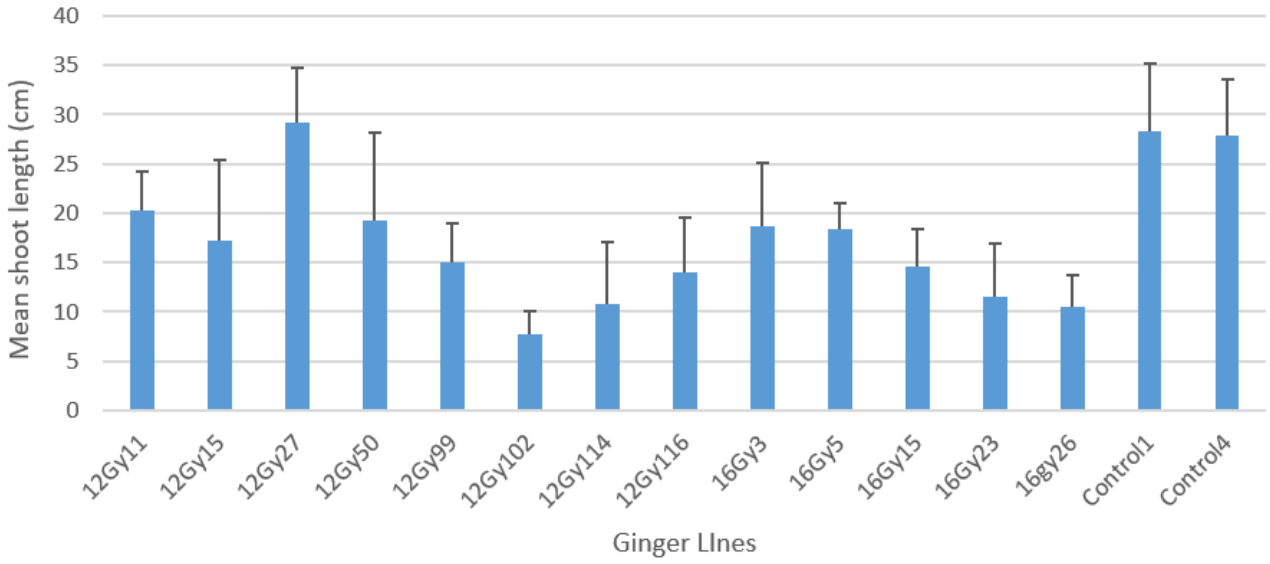

Figure 11: Mean shoot lengths of selected ginger lines versus controls.

as the first tuberization event from TC plants and not from single buds. These rhizomes from TC explants are therefore expected to be smaller.

The natural growing conditions with shorter than 12-hours photoperiod for part of the year also account for the small size of the rhizomes. Senescence halts the development of the rhizomes where there is no control of decreasing natural day length. Marsh et al. [28] has cited senescence within months of transplanting for reduced rhizome size.

The earlier expression of excessive production of roots in ginger is a desirable phenomenon, since this is correlated to high rhizome mass [25]. Reportedly, the root mass increases with pot size. Like rhizome size this must be verified by experimentation since there may be other factors and has not always s been the case [28]. The observations of mean root mass from 1.4 ± 1.3 to 13.59 ± 2.31g compares well with Rezaei et al. [25] 2.65 g in 10 L pots. SRC pots were half that size.

The reported effects of Gamma radiation on rhizome germination and sprouting have largely been inhibitory [17,29,30]. If not observable at low dosages the probability increased with higher dosage [31,32]. The research findings on these irradiated lines at SRC has shown that the all the rhizomes from irradiated mutant lines showed higher germination and sprouting rates. The difference with means of the 12 Gy lines was statistically significant. This communication may be one of the rare observations of an enhancement in germination and sprouting rates from Gamma radiation treatment.

It is interesting that while there was no delayed germination or sprouting, yet reduced shoot length was observed. It is a reasonable assumption that the final shoot length was not to due any early onset growth problems. It may be too early and premature to speculate on the induction of changes in final shoot length, but stunted growth and dwarfism have been attributed to radiation [13,30]. The claim however remains that at 60 days the shoot length of all mutant ginger lines were lower than the control and the 12 Gy means were significantly lower the controls.

Conclusion

The study could have investigated more physical parameters including phenolic compounds in leaves and rhizomes. As a preliminary study non invasive basic data has been useful and the low availability of observations per lines was a constraint. However, the main research question of the significance of radiation on yield and germination has been answered with additional information provided. There is now more confidence that at SRC radiation and dosage effects are not responsible for an apparent nonperformance of mutant ginger lines. On the contrary, the radiation appears to be associated with increased germination and sprouting. There are now opportunities to validate claims made of induction of morphological changes in the rhizomes, enhanced germination and possible stunting or dwarfism in one mutant lines.

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