



The effect of gamma rays irradiation to morphological and agronomical character of local Samosir shallot (*Allium cepa* var. *ascalonicum*)

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Abstract

Gamma rays radiation has been widely used to create variation in gene pools of crop plants. The effects of gamma irradiation on cytological characteristics vary from species to species and among different genotypes within the same species. The aim of the research was to investigate the variations of morphological and agronomic characters of the first generation local Samosir shallot bulbs irradiated at various doses of gamma radiation (0, 1 to 20 Gy with interval 1 Gy). The results indicated that doses of gamma irradiation had significant effects on plants growth and yield of local Samosir shallot. There were differences between regenerated plants growth and yield from irradiated bulbs and control (unirradiated). Overall shoot length and leaf number, tiller number and bulb yield, decreased significantly as the radiation dose increased.

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Introduction

Samosir shallot (*Allium esculentum*) grow in highland surrounding Toba lake, was widely used for culinary purposes. It is very popular in North Sumatra and in a great demand for having typical and pungent scent, more red shiny color, more spicy and less water content although smaller bulb size than other varieties of shallots. However the productivity of this shallot is very low about 1 to 4 ton/ha with the small bulb size, much lower than national shallot productivity which about 9 ton/ha (Sinar Tani, 2012).

In the past several years, because of low local Samosir shallot productivity, most of the farmers in the region has been using other domestic varieties to be cultivated such as Bima Brebes, Kuning, Sumenep, Ampenan, Maja Cipanas, and some other varietas from abroad such as India, Thailand and Vietnam. In the several districts in the surrounding area, that were used to be the centers of shallot cultivation have now converted into coffee cultivation. To avoid the extinction of local Samosir shallot, breeding activities are necessary to produce shallot cultivars that could adapt and yield well in lowland as well as in high land Samosir. However, genetic improvement by hybridization is difficult to perform because shallot is an out crossing and highly heterozygous crop (Eady, 1995).

The lack of inbred lines also makes it difficult to perform genetic linkage analysis in onion (Cramer and Havey, 1999). In addition, local Samosir shallots is not planted using botanical seeds. Alternatively, Samosir shallot breeding can be done by using mutation induction. The induction of mutations has been a successful approach to generate new variability in other crops where natural genetic variation is limited and insufficient.

Induced mutagenesis is an established method for plant improvement, whereby plant genes are altered by treating seeds or other plant parts with chemical or physical mutagens.

Mutation induction is very useful for plants facing problem that land race for hybridization is unavailable. It can increase the genetic diversity of plants (Van Harten, 1988).

Induced mutations serve as a complementary approach in genetic improvement of crops (Mahandjiev *et al.*, 2001).

Mutagenesis has already been used to improve many useful traits affecting plant size, flowering time and fruit ripening, resistance to pathogens, resistance to salinity, fruit, leave and flower color, self compatibility, and self thinning.

It can be done by using a chemical or physical mutagen. One of the most effective physical mutagens to create genetic variation in plants is by using gamma rays irradiation (Human, 2003). Gamma radiation interferes with the process of cell division, resulting in cytological abnormalities and in a reduced frequency of dividing cells, which is ultimately reflected in reduced seedling growth and other morphological aberrations (Amjad and Anjum, 2002). Gamma rays are known to influence plant growth and development by inducing cytological, genetical, biochemical and physiological changes in cells and tissues (Gunckel and Sparrow, 1961).

Harahap (2005) stated that cells that grow well after irradiated experiencing changes in physiological and genetic. The changes could produce superior to the previous crops.

Gamma rays has been widely used for producing mutations in crop plants, and frequently used to create variation in gene pools of crop plants. The effects of gamma radiation on cytological characteristics vary from species to species and among different genotypes within the same species. Din *et al.* (2003) studied the effect of gamma irradiation on different wheat varieties at seed irradiation dose of 10, 20, 30 and 35 krad.

A higher dose of 30 and 35 krad created some abnormalities in plant types for example, a tiller having two ears attached with each and/or prevalence of sterile ears etc.

A lot of work has been carried out on the beneficial effects of gamma irradiation in improving the crops, however for my knowledge, there were very few report informing about impact of gamma irradiation on shallot mutant.

The aim of the research was to investigate the morphological and agronomical characteristics of the first generation treated local Samosir shallot by gamma rays.

Materials and methods

Sixty four (64) dry bulbs 2,5 months after harvest collected from Samosir farm with the weight ranging from 1,3 to 1,7 g by doses of irradiated and non-irradiated local Samosir shallot were packaged in 0.1 mm thick paper bags of 10 cm x 22 cm dimension and sealed. The bags were subjected to gamma rays irradiation in irradiator Chamber 4000 A with Cobalt-60 source at National Atomic Energy Agency, by exposing them to gamma irradiation with doses ranging from 1 to 20 Gy. Subsequently, the irradiated bulbs along with unirradiated bulbs (control) were planted on the field condition during April up to June 2014 for M₁V₁ generation.

The land was cleared and hoed until crumbly. Compost was applied a week before planted with dosage 20 ton per ha, mixed with the soil. Bulbs were planted on 4 rows plot of 5 m x 1.2 m with a spacing of 20 cm x 20 cm, without replication. Plants were watered twice a day, morning and evening for 2 weeks, then once a week until plants six weeks old. Manual weeding was carried out every week. Fertilizer (NPK 15:15:15) was applied twice to each plant, two and four weeks after planting using ring method of application.

The study of morphology was began when the plants were 14 days old (two weeks after planting). Plants are classed as "abnormal" when the plant fails to develop normally because of previous damage to the treated bulb, or when the development as a whole plant is weak or out of the proportion compared to those of normal plants planted at the same time and the same condition. Variabels observed were the number of alive plants, the number of abnormal plants, the average of shoot length, tiller number, bulb number per plant, fresh weight per plant, dried weight per plant, and bulb diameter. The observations on shoot length and leaves number were measured every week began when the plants were 2 weeks until 6 weeks after planting for both control and treated plants.

Shoot length was calculated by measuring the length (cm) from bulb-leaves junction to the shoot tip. The calculation of tiller number began when the plants were 4 weeks old up to 6 weeks. Meanwhile, bulb numbers, weight of fresh bulb per plant were observed at harvest time and dry bulbs were observed 15 days after harvest. The differences between treated plants growth and yield and the control were analyzed by using t-test.

Result and discussion

The plants were evaluated two weeks after planting, and the numbers of alive plants, apparently normal and abnormal plants were recorded for each radiation dosage (Table 1). The results showed that the numbers of alive plant were significantly affected by dosage of irradiation.

The variations on the percentage of alive plants were begun to evaluate at the second week after planting. At the second week after planting, percentage of alive plants up to dosage 17 Gy were found above 50 %, however at the third week, alive plants irradiated at 14 Gy above began to decreased and at the fourth week, whole irradiated plants at 13 Gy above were dead. At the fourth week, alive plants gradually decreased begun from dosage of 7 Gy above. At sixth week, only 14 % of plants irradiated with 12 Gy gamma rays were alive.

Similar results were reported by Dwiatmini *et al.*, (2008) in irradiated *Etilingera elatior* seeds with gamma rays.

They found that the higher dosage of irradiation, the less alive plants, and above dosage 60 Gy no plant found alive. Amjad and Anjum (2002) also reported that seedling growth of irradiated seed onions was reduced severely with an increase in irradiation dose up to 40 krad. Kumar and Dwivedi (2015) observed germination percentages of seeds of three varieties (AA-1, AA-2, and GA-1) of *Trachyspermum ammi* (L.) Sprague (ajwain) that were gamma-irradiated at 100, 200, 300, 400, and 500 Gy by a 60 Co source significantly decreased along with increasing doses of gamma rays.

Table 1. Effect of gamma irradiation on the number of alive and abnormal plants two weeks after planting.

Dosage of Irradiation (Gy)	Number of planted irradiated bulbs	Number of alive plants (WAP)					Number of Abnormal plants (WAP)			
		2	3	4	5	6	3	4	5	6
0	64	62	64	64	64	64	0	0	0	0
1	64	64	64	64	64	64	0	0	0	0
2	64	63	64	64	64	63	0	0	0	0
3	64	59	64	64	64	64	0	0	0	0
4	64	62	63	64	64	63	0	0	0	0
5	64	60	63	63	63	63	0	0	0	0
6	64	58	61	62	61	61	0	0	0	0
7	64	63	64	64	63	58	0	0	0	0
8	64	63	64	63	62	49	0	0	0	0
9	64	57	63	63	57	43	4	0	0	0
10	64	54	63	61	50	26	18	9	2	3
11	64	61	61	58	39	21	27	17	7	0
12	64	59	57	35	16	9	36	15	6	1
13	64	46	51	X	X	X	49	X	X	X
14	64	51	37	X	X	X	32	X	X	X
15	64	43	39	X	X	X	29	X	X	X
16	64	34	28	X	X	X	27	X	X	X
17	64	40	24	X	X	X	24	X	X	X
18	64	23	16	X	X	X	16	X	X	X
19	64	7	4	X	X	X	4	X	X	X
20	64	13	7	X	X	X	7	X	X	X

Normality of plants were also significantly affected by dosage of irradiation. Began from dose of 8 Gy, numbers of normal plants decreased, while numbers of abnormal and weak plants increased. Some irradiated plants at high dosages, leaves became curly, thinner, shorter and smaller. Some bulbs irradiated at high dosage didn't grow at all, some only produced 2 (two) short leaves from beginning until dead, and most of the bulbs irradiated at highest dosage 19 and 20 Gy become sandbar, however overall there was no changing in leave colour except in 2 plants irradiated with dosage of 5 Gy, the colour became light green. Kawamura *et al.* (1992) observed that rice seeds irradiated with gamma rays at 50 krad or more, resulted in reduced root/ length. Amjad and Anjum, (2002) also found that the number of normal onion (*Allium cepa* L.) cv. Ailsa Craig seedlings decreased and that of weak normal and abnormal seedlings increased with the increase in radiation dose. The result is also in line with the report in maize,

the number of abnormalities increased as the dosage of irradiation increased (Viccini and Carvalho, 2001). However, the results quite different from Halim *et al.* (1989) that exposed wheat seeds (C.V. Giza 157) to 0, 2, 4, 8, 16, 32 and 64 krad gamma -irradiation.

They found that germination, plant growth and yield parameters were increased after 2–8 krad seed treatments whereas higher radiation doses decrease all the studied parameters. Previous study revealed that radiosensitivity of local Samosir shallot on gamma rays irradiation was 11,6 Gy. It was found that when the plants 5 weeks old, at dose of irradiation above 9 Gy they still grew, however depressed severely and produced abnormal plants, expressed by stagnation growth, and leaves became thinner and shorter (Mariati *et al.*, 2015).

This suggests that dose of irradiation affected genetic materials resulting in altered morphologi of the plants.

Table 2. Mean shoot length of 2, 3, 4, 5 and 6 week plants after planting.

Dose	Shoots length (cm)				
	2	3	4	5	6
0 Gy	18,63±5,60	24,17±5,63	27,11±3,24	28,67±3,57	29,25 ± 6,10
1 Gy	18,64± 4,22	24,13±3,15	25,60**±2,59	26,35**±4,41	26,87** ±4,45
2 Gy	17,71±5,37	23,37±3,52	25,75*±2,87	26,35**±4,41	26,68**±4,57
3 Gy	15,74**±5,73	21,21**±4,29	24,57**±2,94	26,60**±3,48	26,53**±3,98
4 Gy	15,67**±6,01	21,65*±4,69	23,61**±4,61	25,58**±2,80	26,50**±5,87
5 Gy	16,56*±3,49	20,93**±4,08	22,99**±4,21	26,17**±4,05	23,27**±5,39
6 Gy	15,31**±5,32	18,92**±5,62	21,04**±4,77	22,59**±5,57	20,58**±6,37
7 Gy	13,46**±4,08	17,56**±2,92	19,71**±3,08	21,08**±5,97	18,46**±7,03
8 Gy	10,79**±4,41	14,19**±3,57	16,82**±3,58	19,92**±4,71	14,43**±8,45
9 Gy	9,51**±4,68	11,93**±5,06	15,72**±4,94	17,87**±4,64	13,30**±10,10
10 Gy	5,92**± 3,58	7,95**± 4,99	10,45**±5,83	15,68**±7,06	5,05**± 7,55
11 Gy	5,33**± 4,23	6,95**± 4,81	9,01**± 6,22	10,64**±8,10	4,65**± 7,37
12 Gy	4,02**± 3,90	4,34**± 3,90	4,25**± 5,67	7,25**± 7,25	1,64**± 5,09
13 Gy	1,55**± 3,02	2,39**± 4,29			
14 Gy	1,32**± 2,42	2,70**± 4,78			
15 Gy	1,21**± 2,40	2,57**± 2,67			
16 Gy	0,72**± 2,13	1,02**± 1,48			
17 Gy	0,68**± 2,04	0,95**± 0,88			
18 Gy	0,44**± 2,00	0,78**± 0,73			
19 Gy	0,31**± 1,99	0,35**± 0,19			
20 Gy	0,35**± 1,99	1,75**± 2,50			

*.**: Significant at 5 and 1% level of probability at t test analysis.

Table 3. Leave number of 2, 3, 4, 5 and 6 weeks plants after planting.

Dose	Leave number (WAP)				
	2	3	4	5	6
0 Gy	11,19±3,00	12,98±3,32	16,50±3,96	21,2 ±12,8	18,83±6,26
1 Gy	10,77±2,14	12,73±2,68	14,77**±4,22	15,06**±5,59	14,67** ±4,96
2 Gy	10,48±2,46	12,97±2,94	15,64±4,53	16,14**±5,36	14,44** ±5,03
3 Gy	10,14* ±3,20	12,09*±3,01	15,06*±3,84	16,63*±5,19	14,81**±5,17
4 Gy	10,18*±2,64	12,73±2,63	15,97±3,68	17,20*±5,72	15,38**±6,70
5 Gy	11,32±2,76	12,79±3,78	16,10±4,20	16,90*±5,83	13,11**±4,90
6 Gy	9,91*±3,10	12,87±3,18	14,98*±3,67	14,82**±5,01	12,56**±4,39
7 Gy	9,89*±2,99	12,58±2,61	13,88**±3,69	11,84**±5,11	10,90**±3,73
8 Gy	9,52**±2,54	12,52±3,44	14,67**±4,51	11,74**±4,39	10,26**±4,16
9 Gy	9,60**±2,77	11,62*±3,61	14,52**±3,64	12,79**±5,23	11,84**±4,04
10 Gy	8,16**± 2,87	9,63**±3,26	12,66**±4,57	10,20**±5,00	10,05**±4,08
11 Gy	7,69**±3,19	10,02**±4,06	10,84**±3,98	9,81**±3,57	7,21**±2,90
12 Gy	6,21**±3,56	7,58**±4,69	9,71**±4,44	9,38**±3,96	6,63**±3,38
13 Gy	4,46**±2,68	4,96**±3,76			
14 Gy	3,22**±2,50	4,32**±3,41			
15 Gy	4,30**±2,95	5,46**±4,19			
16 Gy	2,79**±2,51	2,76**±2,26			
17 Gy	2,32**±1,60	2,83**±2,08			
18 Gy	2,14**±1,42	2,31**±1,35			
19 Gy	2,00**±0,82	1,00**±1,00			
20 Gy	1,60**±1,26	2,14**±1,07			

*.**: Significant at 5 and 1% level of probability at t test analysis.

The variation in agronomical characters were also determined, the differences between treated plants growth and the control were analyzed by using t-test. The results showed that gamma rays irradiation significantly affect the growth of local Samosir shallot.

Shoot length, leaves number and tiller number were begun to evaluate two weeks after planting up to six weeks. Mean shoot length, leaves number and tillers number of the second, third, fourth, fifth and sixth week plants are presented at Table 1, 2, and 3.

Tabel 4. Mean Tiller number of 4, 5 and 6 week plants after planting.

Dose	Tiller number (WAP)		
	4	5	6
0 Gy	4.88± 1,05	5.05± 1.19	5.47± 1.27
1 Gy	4.34**± 1,14	4.55± 1.19	4.92± 1.29
2 Gy	4.39*± 1.09	4.78± 1.12	5.27± 1.19
3 Gy	4.11**± 0.89	4.69± 1.01	5.11± 1.22
4 Gy	4.42*± 1.02	4.98± 0.98	5.13± 1.25
5 Gy	4.48*± 1.15	4.94± 1.45	5.17± 1.35
6 Gy	4.47*± 1.05	4.84± 1.33	4.80**± 1.19
7 Gy	4.44*± 1.11	4.69± 1.31	4.59**± 1.19
8 Gy	4.33**± 1.26	4.37± 1.71	4.67**± 1.69
9 Gy	4.03**± 1.41	4.21**± 1.60	4.53**± 1.33
10Gy	3.37**± 1.26	3.48**± 1.34	3.84**± 1.30
11Gy	3.21**± 1.02	3.03**± 0.97	3.75**± 1.39
12Gy	2.97**± 1.09	2.97**± 1.09	2.67**± 1.03

*.**: Significant at 5 and 1% level of probability at t test analysis.

Tabel 5. Mean yield of fresh and dry bulbs per plant.

Dose	Fresh weight (g)	Dry weight (g)
0 Gy	17,60±8,03	15,84 ± 7,84
1 Gy	17,00±14,1	14,20 ± 7,84
2 Gy	16,27±7,40	14,82 ± 7,03
3 Gy	15,24±8,23	13,86 ± 7,42
4 Gy	15,05±6,27	13,83 ± 5,82
5 Gy	7,49±**3,63	6,72 ± **3,46
6 Gy	6,68±**3,48	5,89 ± **3,21
7 Gy	3,00±**1,48	2,43 ± **1,43
8 Gy	3,91±**0,93	3,214 ± **0,85
9 Gy	4,23±**2,46	3,64 ± **2,27

*.**: Significant at 5 and 1% level of probability at t test analysis.

The relationship between dose of irradiation and shoots length at first generation, sixth week after planting is presented at Figure 1. In this study, it was found that dosage of irradiation influenced the shoot length of shallot. Gamma rays irradiation caused reductions in overall treated shoot length compared to unirradiated shoots. The higher dose of gamma irradiation the shorter plant length. Even though the shallots irradiated to a dose of up to 9 Gy performed a good growth viability, but the growth of irradiated plants even at the lowest dose compared to control plants seemed depressed/inhibited,

this was indicated by the length and leaves number of irradiated plants that were shorter and fewer than control plants, however statistically there was no difference between mean shoot length of unirradiated and irradiated plants with dosage 1 and 2 Gy at 2nd and 3th weeks after planting (Tabel 1). The performance of 2 and 4 weeks after planting irradiated plants could be seen at Figure 2 and 3. Similarly results wherein plant shoot length was inhibited even at low dose of radiation has been reported in onion (*Allium cepa* L.) cv. Ailsa Craig (Amjad and Anjum, 2002).

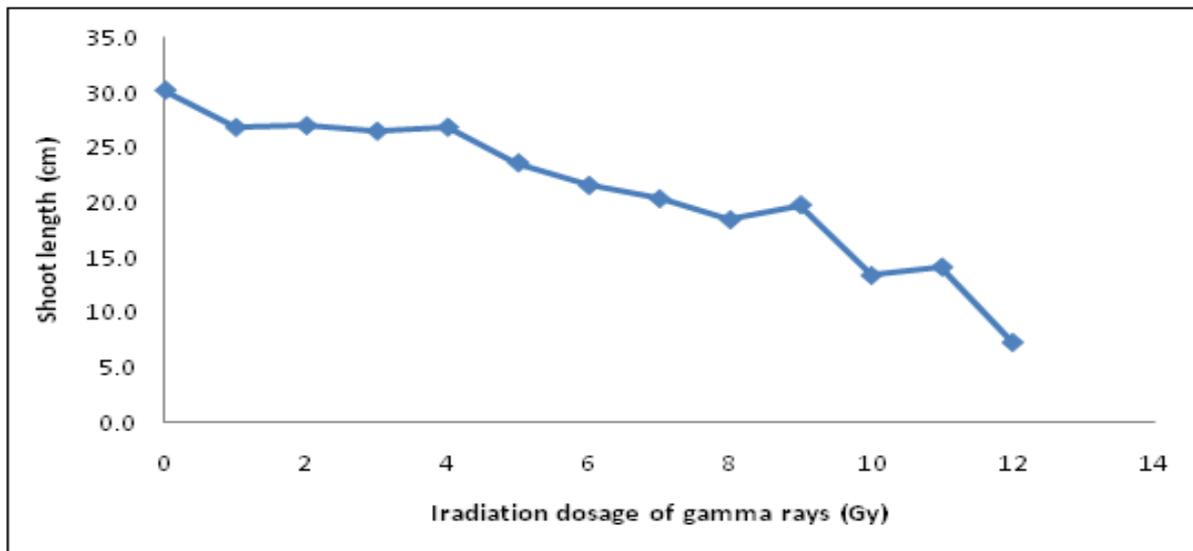


Fig 1. The relationship between dose of irradiation and shoots length at first generation sixth week after planting.

This study also revealed that gamma rays irradiation caused reductions in leave number of irradiated plants compared to unirradiated leave number at the first generation.

The variations on the leave number at the second week up to six weeks after planting could be seen at Table 3. At the second week after planting, leave number produced by irradiated plants with dosage 1, 2, and 5 Gy were not different from leave number produced by unirradiated plants, however, at the third week the decrease of leave number very significant produced by the plants irradiated at 10 Gy above, and at the six week after planting, the reduction of leave number was very significant produced by the irradiated plants even at the very lowest dosage.



Fig. 2. The peromance of 2 weeks after planting irradiated plants along with control plant (o).

The relationship between dose of irradiation and tiller number is presented in Table 4. It can be seen that at the fourth week, whole irradiated plants produced fewer tiller number than unirradiated plants except.. At fifth week after planting there was no difference between tiller number plants irradiated with 1 Gy until 8Gy and tiller number of unirradiated plants. Meanwhile, at sixth week after planting tiller number of the plants irradiated with dosage 1 up to 5 Gy increased slightly and no significantly different from the tiller number produced by control plants. In addition, whole irradiated plants at dosage 6 Gy above have smaller leave size than leaves of control plants.



Fig. 3. The pperformance of 4 weeks after planting irradiated plants along with control plant (o).

According to Van Harten (1998), physiological damages usually occurred at the first generation of irradiated plants and are not heritable traits. Gamma rays produce free radical electron that cause cell damaged. Broertjes and Van Harten (1988) said,

generally irradiation could cause the physiological damages such as inhibit cell division, death of cell, induce mitosis activities, growth rate and genetic changes on the plants. Gamma ray always produces free electrons radical and resulting in damage to the cells.



Fig. 4. The performance of bulbs yield per plant from irradiated bulbs with dose of 1 to 9 Gy along with control (unirradiated) plants.

Similar to other parameter data, it appeared that bulbs irradiated at higher doses suffered greater losses than those irradiated at the lower dose (Fig 4.). Statistically there was no significantly difference on fresh and dry weight bulbs between unirradiated plants and irradiated population treated with dosage at 1 up to 4 Gy gamma rays. However, fresh and dry weight of population irradiated at 5 Gy up to 9 Gy decreased sharply and no bulb produced by irradiated population at 10 Gy above. Similar results were reported by Hanafiah *et al.* (2010) in irradiated *Glycine max* (L.) Merr., var. Argomulto seeds with gamma rays. They reported that phenotypic variations that occur on M_1 plants were caused by changes due to gamma ray irradiation, which affects plant growth, development and production.

Conclusions

Gamma rays irradiation in low doses induced agronomic variations of local samosir shallot at first generation, e.g., shoot length, leaves number, tillers number and bulb yield. As the irradiation dose increased, the shoot length and leaves number decreased. However, shoots length,

leaves number and tillers number of irradiated plants at dose of 9 Gy, increased slightly over those irradiated at dose of 7 and 8 Gy. Gamma rays irradiation affected shallot physiology that were shown by significantly differences between the mean agronomical characteristic of irradiated and control plants.

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