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Mutagenic Effects on the Growth, Reproductive and Yield Parameters of *Praecitrullus fistulosus*

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Abstract. The aim of this study was to modify the growth, reproductive and yield parameters of *Praecitrullus fistulosus* by mutagenesis. The seeds of the plant were treated with chemical mutagens including ethidium bromide and colchicine in concentration of (0.05 and 0.10%) and (0.01 and 0.02%), respectively. Seeds were also treated with UV rays at periods of 1 and 2 h and X-rays for 75 KeV at periods of 5 and 10 sec. The growth features were observed at an interval of one week till the 11th week of their growth period. The data showed that seed germination value and lethality (%) of plants were 80 and 75%, respectively and highest in control plants. The time of seed germination (1.6 days) was least in plants treated with X-rays (10 sec). The mutation frequency (80%) was found to be highest in X-rays (10 sec) and colchicine 0.02%. The vegetative growth parameters such as stem length (77 cm), length of leaves (6.6 cm), average number of leaves (36) and leaf surface area (49.6 cm^2) was highest against ethidium bromide 0.01%, colchicine 0.02%, ethidium bromide 0.10% and UV rays 1 h, respectively. While the average diameter of stem of control plants was highest (49.6 cm^2). The minimum flowering time (31 days) and fruiting time (42.5 days) were observed in ethidium bromide 0.05% and colchicine 0.01% treated plants. The highest number of fruits (4) was observed in colchicine 0.01% treated plants. In conclusion, seeds of *P. fistulosus* treated with ethidium bromide and colchicine caused positive impact on growth, reproduction and yield attributes as compared to UV and X-rays treatments.

Keywords: mutagenic effects, physical mutagens, chemical mutagens, *Praecitrullus fistulosus*

Introduction

Plants are rich source of secondary metabolites that are known to play major role in survival and adaptation of plants to their environment (Bourgaud *et al*., 2001). There are about 200,000 plant secondary metabolites that provide defense mechanism to the plants (Adamu and Aliyu, 2007). World population is increasing at an alarming rate therefore, the food demands have overcome the available land resources. Along with other food sources, vegetables are also considered as alternate inexpensive source of energy for the population of developing countries (Akwaowo *et al*., 2000). Vegetables are rich in biochemicals and nutrients like carbohydrates, protein, vitamins, ascorbic acid, calcium, iron and substantial amount of trace elements (Jimoh and Oladiji, 2005; Prakash and Pal, 1991).

Praecitrullus fistulosus is an annual Cucurbitaceae plant (Tyagi *et al*., 2012) that has been cultivated in Asia since earlier periods and grown mainly in India and Pakistan (Levi *et al*., 2010). *P. fistulosus* is locally called 'tinda' that has tender fruits which are picked during immature stage and used as cooked vegetable (Sujatha and Seshadri, 1989). *P. fistulosus* plant is also rich in many nutritive properties (Gautam *et al*., 2011), anti-inflammatory, anti-tumorous, antidiabetic, antioxidant, anthelminthic, and other therapeutic potential for microbes as an antibacterial agent (Dixit and Kar, 2010).

Food and crop production should be increased at least at the same rate or even much faster rate than world population. In order to improve crop production and enhancement of their economic and agronomic characteristics, mutagenesis can be used (Tah and Roychowdhury, 2011). Mutagenesis is widely used method for crop improvement and variety production (Chopra, 2005). More than 70 decades, mutagenic agents have been employed to induce many different phenotypic traits and variation in plants (Kharkwal and Shu, 2009). Treatment with mutagens may break or rearrange chromosomes by modifying order of genes depending upon the nature of mutagen used in experimental study and do not show any phenotypic expression (Adamu and Aliyu, 2007). Different chemical and physical mutagens are used to induce mutation. Colchicine (COL) is a compound that can cause dynamic instability in

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microtubule formation during mitosis (Ravelli *et al*., 2004). Colchicine has been used widely in cultures to change the ploidy level (Van Harten, 1998). Ethidium bromide (EtBr) is an intercalating molecule that binds by intercalation between the base pairs of DNA strands and is extremely useful tool in different techniques (Palchaudhuri and Hergenrother, 2007). The use of radiation on plants is a wide and complex field and has been applied on a large number of plant species. Radiation affects the germination, growth, development, weight and yield parameters of plants (Sameh *et al*., 2006; Song *et al*., 2006). Ultraviolet radiations (UV) are efficiently absorbed by DNA molecules that causes formation of covalent linkages between pyrimidine bases, hence resulting in the formation of photo dimers (Taylor, 2006), causing blockage of replication as well as transcription (Chopra, 2005). UV-C radiations are shortest wavelength (100-280 nm) with the higher associated energy (Katerova *et al*., 2009) and effects extremely germicidal actions on microorganisms in air, on surfaces and in water (Siddiqui *et al*., 2011).

X-rays penetrate deeper in to the tissues (Amano, 2006) and involves in the production of clusters in DNA that causes damages (Sutherland *et al*., 2000). The effect of X-rays dose on animals and plants has been studied by Yang *et al*. (2011). Different growth regulators can be used such as GABA in order to enhance morphological and yield characters (Islam *et al*., 2010) in addition to increased levels of nitrogen gas and zinc sulphate (Asif *et al*., 2013). In Pakistan, a very little work has been done for the improvement of vegetables germplasm. As *P. fistulosus* contains excellent antioxidant activity as compared to other cucurbits along with antidiabetic and anti-cancerous activities. The primary objective of this study was therefore, to determine effect of colchicine, ethidium bromide, UV and X rays on growth, development and yield attributes.

Materials and Methods

The experiments were carried out during April-October 2014 at the experimental field of Institute of Biotechnology and Genetic Engineering, University of Sindh, Jamshoro, Sindh, Pakistan. *Praecitrullus fistulosus* (tinda seeds) were obtained from private seed company, Hyderabad, Sindh, Pakistan.

Treatment of *P. fistulosus* seeds with chemical mutagens. For induced mutation with chemical mutagens, 0.05% (0.05 g/100 mL) and 0.10% (0.1 g/ 100 mL) of ethidium bromide (ACROS) and 0.01% (0.01 g/100 mL) and 0.02% (0.02 g/100 mL) of colchicine (ACROS) were prepared. Briefly, for each treatment fifty healthy, dry and uniform sized seeds were initially pre-soaked in 100 mL distilled water at room temperature for two hours. After removing the excess water, seeds were submerged in 100 mL solutions containing ethidium bromide and colchicine separately for one hour in the dark with gentle shaking at room temperature (Koornneef *et al*., 1982). The treated seeds were washed in running water to remove excess chemical mutagens and were sowed in the soil. The experiment was repeated for two times thus 150 seeds were used for each treatment.

Treatment of *P. fistulosus* seeds with physical mutagens. Seeds were initially placed in a desiccator with 70% glycerol for two days for moisture equilibration to about 15-20%. For each treatment, fifty healthy and uniform sized seeds were packed in paper bags in petri dishes and placed into the irradiator and were exposed to X-rays of 75 KeV at 5 and 10 sec time periods.

For UV rays treatment, fifty healthy and uniform sized seeds of *P. fistulous* were presoaked in distilled water for two hours at room temperature and then seeds were immediately irradiated with UV C rays at time periods 1-2 h with wavelength of 280 nm using irradiation chamber equipped with UV C lamp (Milan Italy model; G15T8; 3.8 J/m^2 at 1 m of distance). After treatment, the seeds were planted in loamy soil and experiment was repeated twice thus total 150 seeds were used for each treatment.

Growth observations. The effect of chemical and physical mutagens on M_1 plants of *P. fistulous* were observed, at an interval of one week during the growth of the plants. The growth parameters including, seeds germination (%), seeds germination time, mutation frequency (%), lethality (%), stem length, leaf length, stem diameter, number of leaves, leaf surface area, flowering time, fruiting time and the number of fruits per plant, were observed during the study.

Germination (%) of seeds. Germination test was carried out by sowing twenty five seeds on each seedbed and two seedbeds for each treatment. Seeds after absorbing moisture produced a normal root and then first leaf within five to ten days period was considered as germinated seeds. The germination value (%) of each control and treated seeds was calculated according to Don (2005):

$$\text{Germination (\%)} = \frac{\text{No. of seeds germinated}}{\text{No. of seeds in soil}} \times 100$$

Seed germination time. Total seed germination time was calculated by using the following formula:

$$\text{Seed germination time} = \Sigma (d \times n)/ S$$

where:

d = days from sowing; n = number of seedlings germinated on day "d"; S = the number of seedlings germinated (max: final day of total germinated seeds) (Ohba and Simak, 1961).

Lethality percentage (%). Lethality % of plants was calculated by using the following formula:

$$\text{Lethality (\%)} = \frac{\text{No. of plants died}}{\text{Total no. of seeds germinated}} \times 100$$

Calculation of mutation frequency (%). Mutation frequency of the ethidium bromide, colchicine, UV rays and X-rays was calculated by the following formula:

$$\text{Mutation frequency (\%)} = V/P \times 100$$

where:

M = mutation frequency; V = viable mutants observed; P = total plants studied (Tah and Roychowdhury, 2011).

Measurement of stem length and diameter. The stem length was measured by using measuring scale or inch tape while the stem diameter was measured by using thread or scale.

Measurement of leaf surface area and number of leaves. The leaves numbers were counted and leaf surface area was measured. Firstly, the petiole was removed from each control and treated as plant leaves. Leaf surface area was calculated by tracing the leaves of *P. fistulosus* onto 1 cm, and 0.5 cm grid paper. For the calculation of surface area the whole blocks or squares located in the outline of leaves were counted and multiplied by the appropriate area of each grid size or square size (like for 1 cm grid paper it was multiplied by 1 cm^2 and for 0.5 cm grid paper it was multiplied by 0.25 cm^2). Then those squares which included just a small part of leaves outline were counted and divided by 2 since only part of the surface was included within the square. Now the whole square values and the partial square values were added up. As the grid size gets smaller, a better estimate of the true leaf surface area is determined. Using different graph sizes is a great lead to get accuracy and precision in surface area measurement (Gerber, 2014).

Harvesting of the fruit. Harvesting of fruit was started at different time periods in 8th week after germination from each treatment. During harvesting mature fruits were cut from the fruit stalk using a knife.

Statistical analysis. The study plan was in randomized complete block design (RCBD) manner. Analysis was done on all the collected data. The mean of triplicates ± standard deviation was calculated by using Microsoft Excel 2010 and used to assess differences between treatments, while the *p*-value (0.05) was calculated by using IBM SPSS statistics 22.

Results and Discussion

Germination, growth and development of treated *P. fistulosus* plants are presented in Fig. 1-2. Analysis was applied to all measured data which showed significant ($p<0.05$) differences in terms of statistical analysis. The seed germination percentage was determined to be highest in case of control plants which showed that mutagenic treatments decreased the germination percentages but the second highest germination percentage (55.0%) was in case of ethidium bromide 0.10% treated plants. Seed germination (%) was found to be highest in case of control which was 80% as compared to other treated plants, seed germination time was highest in case of control which was 3.5 days while the plants treated with X-rays 10 sec took least time which was 1.60 days, mutation frequency (%) was highest in the plants treated with colchicine 0.02% and X-rays 10 sec which were 80%, lowest lethality (%) was in case of colchicine 0.02% and X-rays 10 sec which was 20% but the growth of X-rays 10 sec treated plants was stunted in starting weeks (Table 1).

Mutagenic frequency was found to be highest in the plants treated with colchicine 0.02% as reported by Tah and Roychowdhury (2011) that colchicine mutagenic action on *Dianthus* showed highest mutation frequency. The effect of ultraviolet B radiations on cucumber (*Cucumis sativus*) seeds resulted in decreased features (Yao *et al*., 2005) and almost similar results were observed in the present study with *P. fistulosus*. Increases in temperature have significant impact on the crop production due to global climate change, *Sorghum bicolor* (L). grown under higher temperatures resulted in more seed production and its germination (Prasad *et al*., 2006) as the tinda plants were grown in direct sun exposure.

Ethidium bromide 0.10% treated plants showed the highest value of stem length (77 cm) while the lowest value of stem length was observed in case of X-rays 10 sec treated plants which were 6.13 cm in the 11th week (Table 2). The plants treated with colchicine 0.02% showed the larger leaf length which was 8 cm while the smaller length of the leaves were observed in X-rays 10 sec treated plants which was 4.60 cm (Table 3). Ethidium bromide treated plants showed increase in leaves as the highest number of leaves (36±2.64) per plant were observed in 0.1% ethidium bromide treated plants as compared to control plants (23.0±3.0 leaves per plant) while X-ray treatment significantly reduced the number of leaves (1.66±0.57) per plant after 11th week (Table 4). Thicker stem diameter was observed in control that is 3.1 cm while the least value of stem diameter was in case of X-rays 10 sec treated plants which was 1.13 cm (Table 5).

Other researchers also observed drastic effects of X-rays on plant growth, development and yield attributes. In chrysanthemum and carnation, form and colour in mutant flowers showed drastic effects when treated with ionizing radiations like X-rays and gamma rays (Tanaka *et al*., 2010) that is the reason tinda seeds treated with X-rays 10 sec retarded in their growth features during initial weeks. These results are also in agreement with the results of Ikram *et al*. (2015) that germination, shoot weight and root length was decreased when mungbean seeds were exposed to X-rays for 10 and 20 sec. Our results are also supported by Thapa (1999) that germination and seedling growth of *P. wallichiana* and *P. kesiya* were inhibited with increased exposure time of gamma rays. The largest leaf surface area was observed in case of colchicine 0.02% and similar results obtained in case of 0.1% colchicine treated *Dianthus caryophyllus* plants (Tah and

Table 1. Effect of chemical and physical mutagens on seed germination time, seed germination (%), lethality (%) and mutation frequency (%) of *P. fistulosus* against control.

Parameters	Control	Chemical mutagens				Physical mutagens			
		EtBr (0.05%)	EtBr (0.10%)	Col. (0.01%)	Col. (0.02%)	UV rays (1 h)	UV rays (2 h)	X-rays (5 sec)	X-rays (10 sec)
SGT (days)	3.43	3.11	2.72	2.25	2.20	2.00	1.75	2.00	1.60
SG (%)	80.0	45.0	55.0	40.0	25.0	15.0	20.0	15.0	25.0
L (%)	75.0	33.3	27.7	37.5	20.0	33.3	25.0	33.3	20.0
MF (%)	ND	66.6	54.5	62.5	80.0	66.6	75.0	66.6	80.0

SGT = seed germination time; SG = seed germination (%); L = lethality (%); MF = mutation frequency (%); ND = not determined; EtBr = ethidium bromide; Col = colchicine; UV = ultraviolet rays.

Table 2. Effect of chemical and physical mutagens on stem length (cm) of *P. fistulosus* against control (mean±S.D, $p<0.05$)

Stem length (cm)	Control	Chemical mutagens				Physical mutagens			
		EtBr (0.05%)	EtBr (0.10%)	Col. (0.01%)	Col. (0.02%)	UV rays (1 h)	UV rays (2 h)	X-rays (5 sec)	X-rays (10 sec)
1st week	3.83±0.28	4.00±0.50	3.66±0.25	2.53±0.25	1.90±0.26	2.93±0.40	2.20±0.34	2.20±0.30	3.06±0.11
2nd week	4.80±0.40	4.20±0.45	4.33±0.40	3.10±0.26	2.33±0.37	3.73±0.25	3.20±0.34	2.76±0.50	3.46±0.25
3rd week	6.36±0.45	4.70±0.30	4.70±0.36	3.36±0.35	2.73±0.35	4.20±0.30	3.83±0.35	3.16±0.30	4.36±0.41
4th week	8.03±0.15	6.90±0.20	7.50±0.30	7.43±0.45	5.80±0.20	6.00±0.34	4.96±0.60	3.63±0.11	4.73±0.15
~~5th week~~	~~14.2±0.30~~	~~12.4±0.36~~	~~9.46±0.40~~	~~8.36±0.41~~	~~6.86±0.15~~	~~7.20±0.36~~	~~5.86±0.35~~	~~5.13±0.58~~	~~4.75±0.40~~
6th week	21.3±0.45	18.3±0.26	19.9±0.41	12.4±0.41	11.0±0.90	9.40±0.50	9.03±0.15	5.83±0.70	4.76±0.30
7th week	25.3±0.45	25.9±0.75	23.8±0.49	20.8±0.30	19.9±0.65	10.6±0.56	9.90±0.30	7.10±0.20	5.46±0.56
8th week	30.9±0.65	29.4±0.75	31.7±0.55	28.3±0.76	20.8±0.90	12.9±0.87	11.0±0.35	7.76±0.47	5.73±0.61
9th week	38.5±0.45	34.5±0.85	40.8±0.30	31.5±0.55	28.2±0.76	20.2±0.65	16.3±1.15	9.80±0.45	5.93±0.51
10th week	48.9±1.87	53.3±1.15	71.7±2.02	48.1±0.60	43.2±1.35	29.3±1.63	19.6±1.01	11.5±0.96	6.10±0.45
11th week	49.5±1.11	57.0±0.81	77.0±1.20	51.9±2.00	49.8±1.23	44.1±2.02	37.8±1.76	18.4±1.36	6.13±0.45

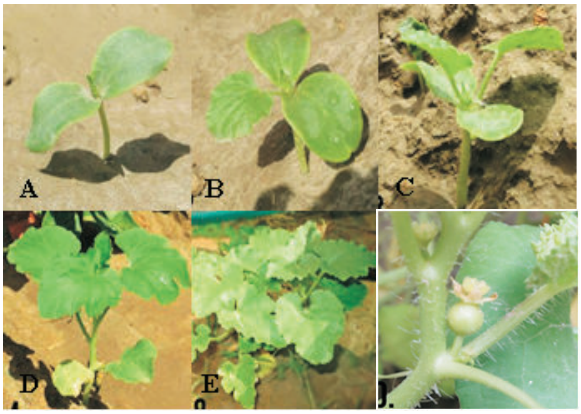

Fig. 1. Germination, growth and fruiting in 0.01% colchicine treated *P. fistulosus*.

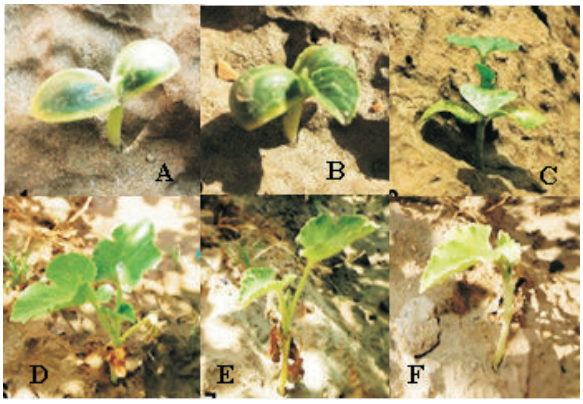

Fig. 2. Germination, growth and fruiting in X-rays (10 sec.) treated *P. fistulosus*.

Table 3. Effect of chemical and physical mutagens on leaf length (cm) of *P. fistulosus* against control (mean±S.D, $p<0.05$)

Leaf length (cm)	Control	Chemical mutagens				Physical mutagens			
		EtBr (0.05%)	EtBr (0.10%)	Col. (0.01%)	Col. (0.02%)	UV rays (1 h)	UV rays (2 h)	X-rays (5 sec)	X-rays (10 sec)
1st week	2.03±0.23	2.23±0.20	2.86±0.30	1.43±0.25	2.00±0.17	2.90±0.20	1.43±0.20	2.73±0.15	2.33±0.15
2nd week	3.46±0.25	2.60±0.10	2.93±0.35	1.96±0.15	2.13±0.20	3.00±0.17	3.30±0.34	3.03±0.05	2.43±0.05
3rd week	4.03±0.11	3.23±0.25	3.26±0.32	4.13±0.20	3.06±0.15	3.10±0.26	4.00±0.20	3.53±0.05	3.90±0.20
4th week	4.13±0.15	4.40±0.17	3.50±0.45	4.36±0.28	3.66±0.25	3.43±0.11	4.16±0.15	3.60±0.10	4.10±0.20
5th week	4.16±0.15	4.56±0.25	3.70±0.36	4.56±0.30	3.93±0.11	3.56±0.11	4.36±0.11	3.73±0.15	4.23±0.20
6th week	4.86±0.15	4.73±0.11	3.86±0.30	4.73±0.25	4.36±0.15	3.90±0.30	4.66±0.11	3.80±0.17	4.26±0.15
7th week	5.40±0.20	5.03±0.15	4.23±0.30	5.23±0.37	4.40±0.17	4.36±0.32	4.86±0.25	4.50±0.20	4.33±0.20
8th week	5.60±0.26	5.16±0.15	4.43±0.40	5.70±0.17	5.03±0.15	4.66±0.35	4.96±0.12	5.00±0.40	4.40±0.20
9th week	6.06±0.15	6.43±0.45	5.86±0.23	5.83±0.05	6.43±0.25	4.96±0.05	5.20±0.30	5.01±0.26	4.46±0.25
10th week	6.26±0.15	6.86±0.15	6.56±0.30	6.03±0.05	6.93±0.15	6.93±0.15	5.36±0.40	6.43±0.35	4.46±0.15
11th week	6.60±0.36	7.83±0.20	6.66±0.28	6.80±0.55	8.03±0.77	7.90±0.40	7.66±0.68	6.56±0.30	4.60±0.20

Table 4. Effect of chemical and physical mutagens on number of leaves of *P. fistulosus* against control (mean±S.D, $p<0.05$)

Number of leaves	Control	Chemical mutagens				Physical mutagens			
		EtBr (0.05%)	EtBr (0.10%)	Col. (0.01%)	Col. (0.02%)	UV rays (1 h)	UV rays (2 h)	X-rays (5 sec)	X-rays (10 sec)
2nd week	2.66±0.57	3.00±0.00	2.33±0.57	3.00±0.00	2.33±0.57	2.66±0.57	2.00±0.00	3.33±0.57	2.33±0.57
3rd week	4.66±0.57	5.33±0.15	4.00±0.00	3.33±0.57	4.00±1.00	6.00±1.00	4.66±0.57	4.33±0.57	4.00±0.00
4th week	7.33±0.57	6.33±0.57	4.66±0.57	4.00±1.00	5.33±1.15	6.66±0.57	5.33±0.57	4.33±0.57	4.33±0.57
~~5th week~~	~~7.66±0.57~~	~~9.00±0.00~~	~~7.33±0.57~~	~~5.00±1.00~~	~~6.33±1.15~~	~~7.00±0.00~~	~~7.33±0.57~~	~~6.33±0.57~~	~~4.33±0.57~~
6th week	10.6±0.57	11.3±1.15	8.66±1.15	7.66±0.57	7.00±1.00	7.66±0.57	8.00±0.00	8.00±1.00	4.33±0.57
7th week	12.3±1.15	13.6±1.52	10.6±0.57	12.0±1.00	8.33±1.15	8.33±0.57	8.33±0.57	8.33±1.15	4.66±0.57
8th week	14.6±1.52	17.3±0.57	14.6±0.57	14.6±1.15	17.3±1.15	8.35±0.57	8.33±0.57	5.66±0.57	2.33±0.57
9th week	25.0±2.00	32.0±2.00	36.0±1.73	21.0±1.00	30.0±1.00	17.3±1.15	23.3±0.57	8.00±1.00	2.33±0.57
10th week	22.0±1.73	28.0±1.00	33.0±3.00	19.3±0.57	31.3±1.52	24.3±1.52	25.3±1.52	8.33±0.57	2.33±0.57
11th week	23.0±3.00	28.3±1.52	36.0±2.64	22.0±1.00	33.0±1.73	29.6±2.08	25.3±0.57	8.66±0.57	1.66±0.57

Roychowdhury, 2011). Our results are also in agreement with Essel *et al*. (2015) that colchicine stimulated the vegetative growth with increased plant height, number of branches and leaves in cowpea. The largest leaf surface area was in case of colchicine 0.02% which was 51.1 cm^2 while the smallest surface area was in

Table 5. Effect of chemical and physical mutagens on stem diameter (cm) of *P. fistulosus* against control (mean±S.D, $p<0.05$)

Stem diameter (cm)	Control	Chemical mutagens				Physical mutagens			
		EtBr (0.05%)	EtBr (0.10%)	Col. (0.01%)	Col. (0.02%)	UV rays (1 h)	UV rays (2 h)	X-rays (5 sec)	X-rays (10 sec)
1st week	0.66±0.05	0.60±0.00	0.50±0.00	0.43±0.11	0.73±0.05	0.66±0.05	0.43±0.11	0.73±0.11	0.66±0.15
2nd week	0.66±0.15	0.63±0.05	0.76±0.15	0.76±0.15	0.76±0.05	0.80±0.17	0.63±0.11	0.86±0.15	0.73±0.05
3rd week	0.80±0.10	0.76±0.05	0.79±0.10	0.76±0.11	0.76±0.15	0.83±0.15	0.63±0.05	0.90±0.10	0.80±0.10
4th week	0.93±0.05	0.93±0.05	1.06±0.11	0.93±0.05	0.93±0.15	0.86±0.15	0.83±0.20	0.90±0.10	0.83±0.11
5th week	1.43±0.25	1.06±0.11	1.16±0.15	1.03±0.15	0.96±0.15	1.20±0.20	0.90±0.10	0.93±0.11	0.86±0.15
6th week	1.83±0.11	1.26±0.30	1.56±0.35	1.60±0.34	1.26±0.25	1.66±0.25	0.96±0.05	1.46±0.15	0.96±0.05
7th week	1.96±0.05	2.00±0.30	1.70±0.43	1.80±0.20	1.26±0.25	1.66±0.25	1.23±0.40	1.63±0.35	1.03±0.15
8th week	2.06±0.20	2.06±0.32	1.83±0.32	1.93±0.11	1.26±0.25	1.70±0.30	1.93±0.45	1.80±0.43	1.13±0.11
9th week	2.40±0.20	2.00±0.17	1.83±0.11	2.06±0.11	1.90±0.26	1.70±0.17	2.16±0.37	2.00±0.26	1.13±0.11
10th week	2.60±0.25	2.16±0.15	2.23±0.20	2.13±0.15	2.36±0.30	2.10±0.36	2.40±0.36	2.05±0.20	1.13±0.20
11th week	3.16±0.20	2.63±0.20	2.83±0.15	2.56±0.11	2.93±0.25	2.20±0.30	2.70±0.20	2.53±0.40	1.13±0.20

Table 6. Effect of chemical and physical mutagens on leaf surface area (cm^2) of *P. fistulosus* against control (mean±S.D, $p<0.05$)

Leaf surface area (cm^2)	Control	Chemical mutagens				Physical mutagens			
		EtBr (0.05%)	EtBr (0.10%)	Col. (0.01%)	Col. (0.02%)	UV rays (1 h)	UV rays (2 h)	X-rays (5 sec)	X-rays (10 sec)
1st week	10.4±0.83	12.9±0.62	10.2±0.15	15.0±0.20	14.9±0.05	15.1±0.41	11.9±0.75	8.76±0.40	7.13±0.32
2nd week	15.5±1.45	17.3±1.19	12.7±0.75	12.7±0.46	22.3±0.41	15.2±1.01	14.5±1.13	12.2±0.68	8.30±0.20
3rd week	17.3±0.50	19.2±0.43	14.0±0.20	24.0±0.63	24.8±0.26	15.7±0.81	17.7±1.11	14.9±0.65	8.97±0.25
4th week	27.6±0.65	29.9±0.36	23.8±0.35	27.1±0.32	28.8±0.32	22.0±0.11	26.7±0.68	20.0±0.20	10.2±0.64
5th week	28.9±0.05	30.3±0.61	25.1±0.32	29.5±0.89	31.2±1.12	27.4±0.68	29.5±0.51	20.1±0.15	10.5±0.63
6th week	29.7±0.50	31.1±0.49	27.3±0.58	28.7±0.64	39.0±0.20	41.3±0.15	38.7±0.87	20.1±0.41	11.0±0.51
7th week	32.2±0.43	34.9±0.17	28.9±0.45	30.9±0.15	39.5±1.30	44.6±1.09	39.6±0.65	21.0±0.05	11.7±0.34
8th week	33.1±0.52	36.0±0.05	29.8±0.15	31.6±0.76	41.1±1.00	46.8±0.76	40.8±0.75	21.2±0.25	11.9±0.40
9th week	35.6±0.55	37.2±0.43	30.6±0.35	34.4±0.45	42.0±0.20	47.6±1.09	43.0±1.69	22.0±0.20	12.2±0.26
10th week	38.4±0.50	39.1±0.32	33.8±0.98	37.7±1.16	48.9±0.85	48.9±1.24	46.0±1.80	22.2±0.26	12.7±0.20
11th week	39.5±0.51	50.8±0.90	34.2±0.92	43.2±2.34	51.1±0.69	49.6±1.47	44.3±1.53	22.4±0.37	12.9±0.05

Table 7. Effect of chemical and physical mutagens on flowering time, fruiting time and number of fruits of *P. fistulosus* against control (mean±S.D)

Parameters	Control	Chemical mutagens				Physical mutagens			
		EtBr (0.05%)	EtBr (0.10%)	Col. (0.01%)	Col. (0.02%)	UV rays (1 h)	UV rays (2 h)	X-rays (5 sec)	X-rays (10 sec)
FLT (days)	44.5±0.70	31.0±1.41	42.5±0.70	38.5±2.12	49.0±1.41	---	---	---	---
FRT (days)	---	---	60.0±1.41	42.5±2.12	69.0±2.82	---	---	---	---
NF	---	---	2.50±0.70	4.00±1.41	2.50±0.70	---	---	---	---

FLT = flowering time, FRT = fruiting time, NF = number of fruits.

X-rays 10 sec which was 12.9 cm^2 (Table 6). Mutagenic effects on plants features may produce resistance in many susceptible crops and their yield improvement (Al-Qurainy and Khan, 2009). Mensah *et al*. (2007) also obtained higher yield or number of fruits in low concentration of colchicine (0.01%) in *Sesame indicum* L. The minimum flowering time was observed in ethidium bromide 0.05% treated plants which was 31 days, while the flowering time increased in case of colchicine 0.02% treated plants which was 49 days but the plants with treatments such as UV (1 h and 2 h) and X-rays (5 sec and 10 sec) did not show any flowering, while fruiting time was minimum in case of colchicine 0.01% that was 42.5 days and maximum in colchicine 0.02% treated plants but the plants treated with ethidium bromide 0.05%, UV (1 h and 2 h), X-rays (5 sec and 10 sec) and control did not show any fruiting. The highest number of fruits was observed in colchicine 0.01% which was 4 in number, while lowest number of fruits was observed in case of ethidium bromide 0.01% and colchicine 0.02% treated plants. On the other hand, control plants were flowered but failed to fruit due to high temperature (Table 7). These results revealed that treatment of *P. fistulosus* seeds with colchicine (0.01 and 0.02%) and ethidium bromide (0.1%) may be beneficial that increased heat tolerance and thus made flowering and fruiting even when temperature increased to 45 °C during the month of June.

These results are in agreement with Dhakhanamoorthy *et al.* (2010) that early flowering and fruit maturity may be due to the physiological changes caused by mutagen. The secondary compounds such as phenolic acids, antioxidants, reducing power, ascorbic acids, etc. were significantly enhanced in colchicine treated *P. fistulosus* plants as compared to other mutagens treated plants (data not shown). The fruits of *P. fistulosus* are used as vegetable while the leaves are the agriculture wastes. The secondary metabolites of the plants have commercial interest both in research and pharmaceuticals for the manufacturing of the effective drugs. The growth features can be improved or modified under the influence of mutagenic treatment. The present study has provided evidence on the induction of mutations causing genetic variability associated with growth, reproductive and yield parameters of tinda (*Praecitrullus fistulosus*). Thus, genetic variability caused by mutation induction can be effectively utilized for modifying mutant strains possessing desirable and novel attributes.

Conclusion

The results of mutagen treatment of *P. fistulosus* revealed ethidium bromide and colchicine treatment of seeds improved germination, growth and yield attributes in high temperature while UV and X-rays treatment showed opposing results of these attributes. Similarly 0.01% colchicine may be used for the improvement of growth, reproductive and yield parameters in *P. fistulosus*.

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