



Effect of Physical and Chemical Mutagens on Mitotic Cell Division Rate (MI) in *Rivinia humilis* L.

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ABSTRACT

Rivinia humilis L. was identified as a vital and more reliable source of red natural dye obtained from its ripened berries (fruits). The germplasm of the plant was subjected to the treatment of physical (gamma irradiation) and chemical mutagens (Sodium azide and Ethyl methanesulphonate) for the enhancement of dye content, by inducing the genetic variability in the genome. In the present investigation, both the chemical mutagens exhibited mito-depressive effect, where the mitotic activities were progressively decreased in all treatment modes. The root-tips of treated seeds exhibited dose/concentration dependent decrease in mitotic activities, in all the three mutagens, however, stimulation was noted with lower doses of gamma irradiation only. The drastic reduction in mitotic activities in root tip cells due to the treatment of different concentrations of both chemical mutagens and higher doses of gamma irradiation indicated the high degree of sensitivity of the genome of *Rivinia humilis* L. All concentrations of both the chemical mutagens resulted in the suppression of mitotic activities, whereas, only lower doses of gamma rays reported to be promontory in action. Among chemical mutagens, EMS was reported to have more adverse effect on mitotic activities than SA. The adverse effect was observed to be enhanced with the increase in pre-soaking period in both the cases, however, 6h pre-soaking treatment mode of EMS was reported to be more mito-depressive than dry seed and 3h pre-soaking treatment modes. Comparatively, higher doses of gamma irradiation had more adversely affected the mitotic activities than both the chemical mutagens employed in the present investigation. The cytological analysis, in terms of positive and negative responses of all the three mutagens revealed that the genome of the plant is highly sensitive and hence could be used to change the genetic architecture of the plant for obtaining the desired mutants.

Keywords: *Rivinia humilis*, mutagens, mito-depressive, stimulatory, sensitivity.

INTRODUCTION

Rivinia humilis L. is a monotypic genera belonging to the pokeweed family Phytolaccaceae. It yields natural red dye from its ripened berries. The plant is native of tropical America and is listed as a weed in several countries around the world. The plant is occasional weed and is naturalized in Sri Lanka, India and Malaysia (Mathew, 1983). The plant is deliberately brought to India from Florida, strictly for the ornamental purposes and mostly grown in gardens and greenhouses (Naik, 1998). The plant is perennial herb and bears green coloured un-ripped berries. The fully matured ripened berries yield a red natural dye containing red-violet pigment known as rivianin or rivinianin. It has sulphate group attached, and is very much similar to betanin, the pigment found in beet root. It contains red-violet betacyanin derivative, confirmed as betanin 3'-sulphate by Imperato (1975) and orange-yellow betaxanthin derivative named as humilaxanthin by Strack *et al.* (1987). The plant has reported to possess many medicinal properties too. Natural products obtained from different parts of *Rivinia humilis* L. is traditionally used in Jamaica as antidote to poisoning, headache and cold, diarrhea, marasmus and inflammation (Mitchell & Ahmad, 2006). Salyat *et al.* (2001) reported the inhibitory effects of methanolic extracts of the branches of this plant against *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Enterococcus faecium*, whereas, Fathima and Tilton (2012) confirmed the radical scavenging activities of leaf extracts in methanol, and suggested its potent antioxidant activity. The methanolic and aqueous extract of the fruits are tested by Parvatm and Ravishankar (2011) for its antioxidant properties. Khan *et al.* (2013) evaluated the effect of berry extract of *Rivinia humilis* L. on physicochemical properties and acceptability of the product, and observed the retention of 68% of the colour in *Rivinia* banana spread after 6 months of storage at 5°C, without the alteration of product quality. Joseph and Avita (2013) carried out the studies on antimicrobial activities of root and shoot against 10 bacterial and 4 fungal strains and reported the inhibitory effects at all the strains of bacteria.

Mutations are the tools and being used to study the nature and function of genes which are the building blocks and basis of plant growth and development, thereby producing raw materials for genetic

improvement of economic crops (Adamu & Aliyu, 2007). The mutation, in a true sense, leads to loss or gain of function of a gene and that can be handed over to the next generation, if not auto-corrected and when passed through the germ line. These induced mutations led to the change in genetic architecture of plants which are reflected at physiological, morphological and biochemical levels (Aney, 2013a, 2013b). Mutation breeding proved to be an important tool in introducing different desirable characters of agronomic value in various plants, mostly the crop plants.

Cytological analysis with respect to mitotic and meiotic behavior of the chromosomes is the most reliable tool to evaluate the effect and potency of various mutagens. It also provides a clue to assess sensitivity of genotypes of various plants for different mutagens including both physical and chemical mutagens. Irradiations of the seeds with physical mutagens, particularly, the gamma irradiations, induce gross structural changes in the chromosomes and have inhibitory effect on most of the morphological and yield attributing characters. The mutagenicity of different chemical mutagens such as sodium azide (SA) and ethyl methanesulphonate (EMS) to disturb the structural entity of chromosomes, in many plants, is also well established. The chemical mutagens, generally induce point or gene mutations, leading to the base pair substitutions and thus changing the functions of proteins without abolishing them. The mutagenic effects occurred at the chromosomal level leading to the change in genetic architecture of the plant might results in variations at morphological and physiological level. It helps to identify and isolate various mutants for morphological, physiological and different yield attributing and desirable characters. Hence, the cytological analysis with respect to chromosomal aberrations, either in mitosis or meiosis, is regarded as one of the most dependable criteria for estimating the effect of mutagens. It also provides a clue to assess sensitivity of genotypes of various plants for different mutagens. Although *Rivinia humilis* L. is deliberately introduced as non-native naturalized plant in India but is found best suited in Indian agronomic climate. We identified *Rivinia humilis* L. as a reliable source of red natural dye that can provide an opportunity to be an alternative source of natural red dyes which are quite difficult to obtain from the underground parts of the other existing plants..

MATERIALS AND METHODS

The germplasm of *Rivinia humilis* L., in the form of seeds, was procured from five different localities viz., Research field, Department of Botany, RTM Nagpur University, Nagpur; Paradise Nursery, Nagpur; Giripeth and Shantivihar area, Nagpur and from Pauni, Dist. Bhandara. Healthy and uniform sized seeds were selected and exposed to gamma rays with 50, 75, 100, 125, 150 and 200Gy doses. Three different treatment modes viz., dry seed (DS), presoaking in water for 3h (PSW-3H) and 6h (PSW-6H), were used for both the chemical mutagens (SA & EMS). The healthy and uniform sized seeds were treated with both the chemical mutagens for 18h with 0.0075, 0.010, 0.020% of freshly prepared SA and 0.50, 1.00 and 1.50% concentrations of EMS. The treatment was terminated by decanting the mutagen solutions, and the treated seeds were thoroughly washed, several times, with distilled water to remove the traces of mutagen. Twenty seeds from each dose/concentration, along with untreated (control), of both physical and chemical mutagens were kept for germination, on germination paper slots. After initiation of germination, root tips of 1-1.5cm length were cut and fixed between 8-10 am, in freshly prepared Carnoy's fluid (3 absolute alcohol:1 glacial acetic acid) for arresting the mitotic activities, for 24 hours, and finally preserved in 70% ethanol.

The squashes of the root tips were prepared for mitotic studies by first thoroughly washing with distilled water, and then hydrolyzing in 1N HCl for 15 min at 60°C. The hydrolyzed root tips were washed twice in distilled water and kept for mordanting in 4% freshly prepared ferrous ammonium sulphate (iron alum) solution for 5 minutes. The traces of mordant from the root surface were removed by washing three times with distilled water. Subsequently, the root tips were stained in 1% haematoxylin stain for 5 to 7 minutes. Stained root tips were squashed in 45% acetic acid and covered with cover slip. The data on mitotic index and mitotic chromosomal abnormalities were recorded and photographed and were made permanent using different grades of butanol:acetic acids (Darlington & La Cour, 1976). Mitotic indexes (MI), % relative division rate (% RDR) and percentage abnormality were calculated in following ways (Jabee *et al.*, 2008; Aney *et al.*, 2012).

$$\text{Mitotic index (MI)} = \frac{\text{Total number of cells in division}}{\text{Total number of cells scored}} \times 100$$

$$\% \text{ RDR} = \frac{\text{Percentage of dividing cells in roots treated Dose / concentration} - \text{Percentage of dividing cells in root tips of control seeds}}{100 - \text{Percentage of dividing cells in root tips of control seeds}} \times 100$$

$$\% \text{ Abnormality} = \frac{\text{Total number of aberrant cells in the root tips of treated seeds}}{\text{Total number of cells in division (M+A+T)}} \times 100$$

RESULTS

The mutagens used in the present investigation, in past, have been proved to be effective on the mitotic cell divisions in the root tip cells of different plants. The number of dividing cells decreased with the increase in dose/concentrations of gamma rays and both the chemical mutagens (Tables 1-3). As evident from the values of mitotic index (MI) in terms of number of dividing cells (Table 1; Fig. 1) and the % relative division rate (% RDR) (Fig. 2), the mitotic activities in the root tip cells of the seedling of the gamma irradiated seeds was found to be greatly affected. The lower doses (50 & 75Gy) had slightly stimulatory effect on the mitotic process, while the same was found adversely affected at higher doses (150 & 200Gy), where it was almost reduced to half as compared to the control (Table 1; Fig. 1). The lower doses (50 & 75Gy) of gamma rays promoted the mitotic activities from 26.22 to 27.33% as to that of 25.13% in control root tips, whereas, the mitotic activities, was reduced to 19.33 to 11.80%, from the dose 100Gy onward. The positive values of % RDR at lower doses of gamma rays is an indication of the stimulation of mitotic process by these doses. The promotary effects of lower doses of gamma rays improved the % RDR which was 1.46 and 2.97% at 50 & 75Gy, respectively (Fig. 2). Negative values of % RDR, due to certain higher doses (100-200Gy), is an indication of suppression of mitotic process due to the exposure to gamma irradiation. The % RDR at these doses ranged between -7.73 and -17.79% (Fig. 2) that clearly indicated the suppressive effects of gamma rays on the mitotic process, in the plant under study.

Table: 1. Effect of different doses of gamma irradiation on mitotic cell division (MI) and % RDR in root tip cells of *Rivinia humilis* L.

Doses of gamma rays (Gy)	No. of cells scored	No. of cells in division	Mitotic Index (MI) %	Relative Division Rate (RDR) %
Control	2959	743	25.13 ± 0.51	-
50	2993	785	26.22 ± 0.64	1.46 ± 0.24
75	3002	820	27.33 ± 0.56	2.94 ± 0.24
100	2800	542	19.33 ± 0.37	-7.73 ± 1.97
125	2935	525	17.90 ± 0.57	-9.65 ± 0.49
150	2893	391	13.53 ± 0.23	-15.48 ± 2.46
200	2755	325	11.80 ± 0.24	-17.79 ± 1.57

Table: 2. Effect of different concentrations of SA on mitotic cell division (MI) and % RDR in root tip cells of *Rivinia humilis* L. under variable treatment modes.

Concentrations of SA (%)	No. of cells scored	No. of cells in division	Mitotic Index (MI) %	Relative Division Rate (RDR) %
Control (DS)	2698	686	25.55 ± 0.68	-
0.0075 (DS)	2867	643	22.39 ± 0.46	-4.26 ± 0.29
0.010 (DS)	2721	574	21.11 ± 0.38	-5.98 ± 0.67
0.020 (DS)	2739	516	18.82 ± 0.31	-9.06 ± 1.26
Control (PSW-3H)	2517	629	25.55 ± 0.27	-
0.0075 (PSW-3H)	2746	573	22.39 ± 0.55	-5.38 ± 0.90
0.010 (PSW-3H)	2586	471	21.11 ± 0.33	-9.04 ± 0.51
0.020 (PSW-3H)	3107	513	18.82 ± 0.46	-11.26 ± 0.48
Control (PSW-6H)	2865	744	26.06 ± 0.56	-
0.0075 (PSW-6H)	3058	668	21.85 ± 0.12	-5.69 ± 1.24
0.010 (PSW-6H)	2977	518	17.38 ± 0.27	-11.71 ± 1.55
0.020 (PSW-6H)	3057	462	15.11 ± 0.58	-14.81 ± 2.75

Table: 3. Effect of different concentrations of EMS on mitotic cell division (MI) and % RDR in root tip cells of *Rivinia humilis* L. under variable treatment modes.

Concentrations of EMS (%)	No. of cells scored	No. of cells in division	Mitotic Index (MI) %	Relative Division Rate (RDR) %
Control (DS)	2698	686	25.27 ± 0.83	-
0.50 (DS)	2867	643	22.99 ± 0.53	-3.04 ± 0.65
1.00 (DS)	2721	574	20.72 ± 0.29	-5.96 ± 0.65
1.50 (DS)	2739	516	16.78 ± 0.46	-11.36 ± 0.96
Control (PSW-3H)	2517	629	27.79 ± 0.21	-
0.50 (PSW-3H)	2746	573	21.04 ± 0.61	-9.35 ± 0.30
1.00 (PSW-3H)	2586	471	19.06 ± 0.42	-12.09 ± 1.73
1.50 (PSW-3H)	3107	513	15.93 ± 0.65	-16.42 ± 0.42
Control (PSW-6H)	2865	744	28.02 ± 0.36	-
0.50 (PSW-6H)	3058	668	19.35 ± 0.67	-12.05 ± 0.35
1.00 (PSW-6H)	2977	518	16.38 ± 0.22	-16.18 ± 2.14
1.50 (PSW-6H)	3057	462	13.56 ± 0.33	-20.09 ± 0.52

Fig.: 1. Effect of different doses of gamma rays on mitotic activities in root tip cells of *Rivinia humilis* L.

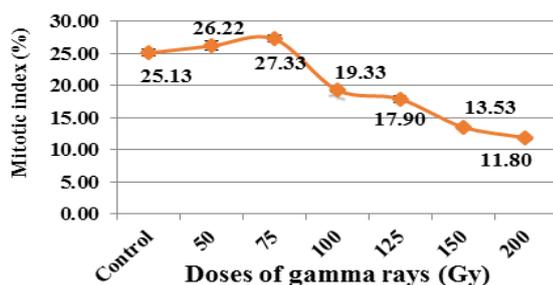


Fig.: 2. Effect of different doses of gamma rays on % RDR of mitotic division in root tip cells of *Rivinia humilis* L.

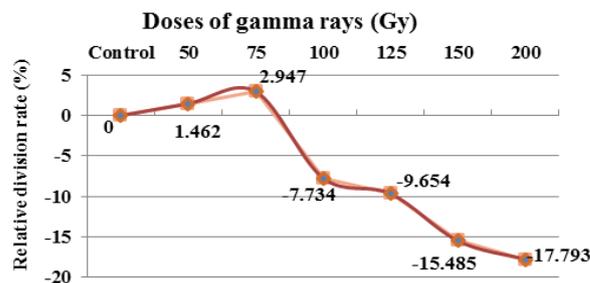


Fig.: 3. Effect of different conc. of SA on mitotic activities in root tip cells of *Rivinia humilis* L., under variable treatment modes.

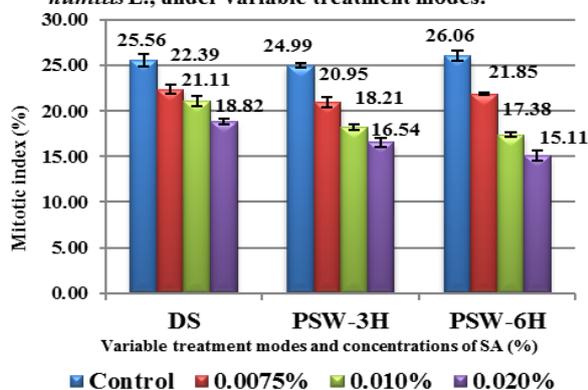


Fig.: 4. Effect of different conc. of SA on % RDR of mitotic division in *Rivinia humilis* L., under variable treatment modes

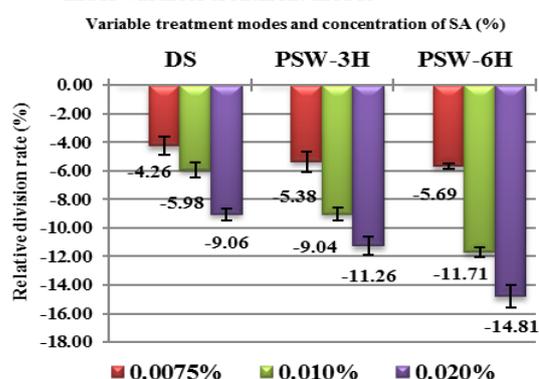


Fig.: 5. Effect of different conc. of EMS on mitotic activities in root tip cells of *Rivinia humilis* L., under variable treatment modes

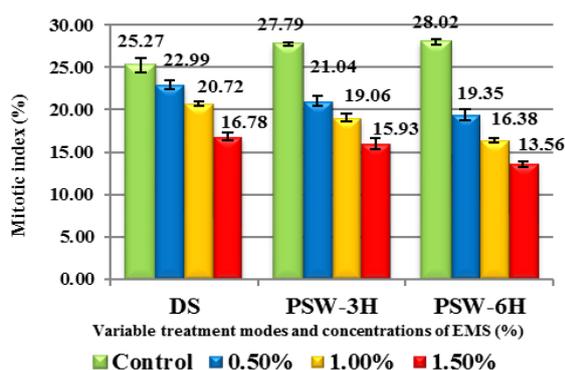
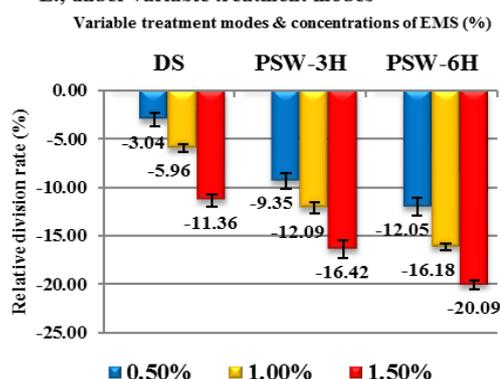


Fig.: 6. Effect of different conc. of EMS on % RDR of mitotic division in *Rivinia humilis* L., under variable treatment modes



Figs: 1-6: 1: Effect of different doses of gamma rays on mitotic cell division (MI) in root tip cells of *Rivinia humilis* L., 2: Effect of different doses of gamma rays on mitotic % RDR in root tip cells of *Rivinia humilis* L., 3: Effect of different concentrations of SA on mitotic cell division (MI) in root tip cells of *Rivinia humilis* L. 4: Effect of different concentrations of SA on mitotic % RDR in root tip cells of *Rivinia humilis* L. 5: Effect of different concentrations of EMS on mitotic cell division (MI) of root tip cell of *Rivinia humilis* L., 6: Effect of different concentrations of EMS on mitotic % RDR in root tip cells of *Rivinia humilis* L.

Both the chemical mutagens, in all treatment modes, exhibited mito-depressive effect as evident from the decrease in mitotic activities in concentration dependent manner (Tables 2 & 3; Figs. 3 & 5). The data on mitotic index displayed in tables 2 and 3 clearly revealed the progressive reduction in mitotic activities in terms of number of dividing cells (MI) with the increment in concentrations of both the chemical mutagens, in all treatment modes. However, higher concentrations of both the chemical mutagens, in all treatment modes, more severely affected the mitotic process than the lower concentrations (Tables 2 & 3). The data on mitotic indices shown in tables 2 and 3 clearly revealed that effectiveness of both the chemical mutagens had been not only enhanced by the presoaking of seeds but also increased with the presoaking duration. The rate of mitotic division in control root tips, in all treatment modes of SA, was almost more or less same, whereas, it was found progressively reduced in the range from 22.39 to 18.82% in dry seed, 20.95 to 16.54% in 3h presoaking treatment mode and 21.85 to 15.11% in 6h presoaking treatment mode (Table 2; Fig. 3). Adverse effect of SA treatment to the seeds, on mitotic process, after presoaking in water, was found to be enhanced and the enhancement was reported to be increased with the increase in presoaking duration. Maximum reduction (15.11%) was observed with 0.020% concentration of SA, in 6h presoaking treatment mode. The concentration dependent reduction in mitotic activities, due to SA treatment, also affected the % RDR, in all treatment modes of SA (Fig. 4). The adverse effect was progressively enhanced with the increment of SA concentration, where, maximum was attend at higher (0.020%) concentration of the applied chemical mutagen. Similar trend of reduction in mitotic activities, due to treatment of mutagen, was also reported with EMS (Table 3; Fig. 5). The % MI, in control of all treatment modes of EMS, was 25.27, 27.79 and 28.02% in dry seeds, 3h and 6h presoaking treatment modes, respectively, whereas, it was reduced in the range of 22.99 to 16.78% in dry seeds, 21.04 to 15.93 in 3h presoaking and 19.35 to 13.56% in 6h presoaking treatment mode of EMS (Fig. 5). Reduction in mitotic activities, due to EMS treatment, affected the % RDR also (Fig. 6) that clearly indicated the suppressive effect of mutagen on the mitotic process. The % RDR was found to be affected less at 50%, moderate in 1.00%, but significantly in 1.50% concentration of EMS, in all treatment modes.

Mito-depressive effects of higher doses of gamma irradiation and different concentrations of both the chemical mutagens is clearly evident from the dose/concentration dependent decrease in rate of mitotic cell divisions in the root tip cells of *Rivinia humilis* L. Among all the mutagens, exposure of seeds to gamma rays was proved to be more effective than both the chemical mutagens, however, the treatment of seeds with EMS was reported to have more adverse effects on mitotic process than the treatment with SA. The presoaking of seeds in water, before the treatment of both the chemical mutagens, enhanced the deleterious effects on the mitotic activities (Tables 1-3; Figs. 1-6).

DISCUSSION

Mito-depressive effects of the mutagens, particularly at higher doses of gamma irradiation and both chemical mutagens were evident from the cytological analysis of the data on mitotic process. The concentration dependent decrease in mitotic index (MI) and percent relative division rate (% RDR), in case of both the chemical mutagens, revealed adverse effect of mutagens on the mitotic process. Mitotic process and the chromosomes were found to be greatly affected by all the mutagens. The enhanced frequency of mitotic aberrant cells and various chromosomal aberrations is an indication of cytotoxic effects of all the mutagens on the genome of plant under investigation. The genetic damage resulted by the induction of disturbances in the mitotic and meiotic cells due to the treatment of mutagens is of great importance since, these genetic damages are handed over to the next generation (Kumar and Rai, 2007). The mitotic index is used as indicators of adequate cell proliferation (Gadano *et al.*, 2002). The stimulation of mitotic activities at lower doses of gamma rays was reported by Sarda Mani and Reddi (1985) in Sorghum, Jayabalan and Rao (1987) in *Lycopersicon esculentum*, Yadao (1987) in *Vigna radiata*, whereas, mito-depressive effects of higher doses of gamma rays and drastic reduction in % RDR was noted by Eroglu *et al.* (2007) in *Hordeum vulgare*, Shukla and Kumar (2010) in *Lathyrus sativus*, Kumar and Srivastava (2010) in Safflower, Aney *et al.* (2012) in two cultivars of *Pisum sativum* and Girija *et al.* (2013) in *Vigna unguiculata*. The concentration dependent mito-depressive effects of SA and EMS, in all treatment modes, in *Rivinia humilis* L. are in

confirmation with the results obtained by Pearson *et al.* (1975) in barley, Choudhary and Dnyansagar (1980) in garlic, Ilbas *et al.* (2005) in barley, Bhat *et al.* (2007) in *Vicia faba*, Kamble and Petkar (2014) and Kamble and Patil (2014) in *Cicer reticulatum* L. However, Dhulgande *et al.* (2015), in chickpea, observed the reduction in mitotic index at lower concentration and stimulation at higher concentration of EMS.

The increase in mitotic index at lower doses of gamma rays can be due to stimulation of mitotic activities and can also be correlated with the increased activities of root apical meristem that finally resulted in the increase in root length. Consequently, the increase in mitotic indices at these doses improved the relative division rate. Similar observation was also noted by Sax (1963). Verma *et al.* (2012) observed the increase in mitotic index values in root meristem treated with EMS in *Catharanthus roseus* and that was attributed to the result of accumulation of C-metaphase configuration at zero hour recovery (Badr, 1983). The stimulation in growth in *Melilotus alba*, at lower doses of thermal neutrons, was observed by Micke (1961) who opined that the low radio-activity in the seeds was possibly responsible for the stimulation of auxin synthesis resulting in the enhancement of mitotic process and finally the growth. The gradual decline in mitotic index and relative division rate at higher doses of gamma rays and at all concentrations of both chemical mutagens indicated the cytotoxic and genotoxic effect of all the mutagens. The decline in the mitotic activities by the mutagen is attributed to chemical changes brought about in the cell (Lea, 1955), acceleration of formation of antimetabolic substances (Rubin and Metlisky, 1958) and blockage of DNA synthesis (Heiner, 1971). The ATP demand of dividing cell is much higher compared to non-proliferating cells. The treatment of SA adversely affects the enzyme induction and that delay in enzyme induction resulting in the deficiency of ATP may be one of the reasons for the decline in mitotic activities (Pearson *et al.*, 1975). Sinha and Godward (1972) correlated the mitotic delay and inhibition to the less acute disturbances caused at the cellular level either to the gene controlling biochemical, physiological processes or chromosomal aberrations. However, Sarda Mani and Reddi (1986) explained the cause of decline in mitotic activities due to mutagen treatment and according to them the treatment of mutagens induces the 'endogenous poisons' inside the cell which

may arise by the breakdown of micro and macromolecules especially enzymes and nucleoproteins in the cytoplasm. These mitotic poisons may cause 'metabolic imbalance' which may interfere with synthesis, state and structure of nucleic acids including physiological effects and structural changes in the chromosomes during cell division. However, Tramontano and Scaloni (1996) observed the necrosis of root in legumes due to higher doses of mutagen and have given the possible reason for modification in mitotic index that, there occurs the accumulation of sodium propionate or sodium butyrate that leads to the inhibition of DNA synthesis (Elena, 2010). The reduction in mitotic index due to treatment of both chemical mutagens could be due to blockage of cells at G₂ phase. The chemical mutagens could be considered analogous to 'prophase poisoning', described by D'Amato (1949, 1952), where some of the cells in early prophase may revert back to the interphase condition, thereby considerably reducing the number of dividing cells. The observation of reduction in mitotic activities and increase in frequency of aberrant cells and chromosomal aberration in both the presoaking treatment modes of chemical mutagens might be due to increase in permeability of cell membrane (Wallis, 1967) and activation of seeds at physiological level (Roychowdhary and Tah, 2013).

Thus, in *Rivinia humilis* L. all the chemical mutagens and gamma rays particularly, at higher doses exhibited the mito-depressive and cyto-toxic effects. The employed mutagens not only affected the mitotic activities in positive and negative ways affected the genome of the plant under study by inducing the various clastogenic and non-clastogenic effects on the chromosomes. The results of the effects of all the mutagens in the present studies, on the mitotic activities and chromosomes clearly revealed that the genome of the plant is highly sensitive to the mutagens used. The mutagenic effectiveness increased with the increase in dose/concentration of all the mutagens leading to the changes in genetic architecture of the plant. It resulted in inducing the genetic variability at the genome level that has been displayed in the variation in plant morphology, chlorophyll, sterility and yield, which can help to identify and isolate the mutants for desirable characters which could be favourably exploited for the improvement of agronomic characters of the plant.

CONCLUSION

The data on effectiveness of different mutagens on the mitotic activities in the treated root tip cells, in the present study, clearly indicated that all the mutagens have deleterious effects on the mitotic cell cycles. The effect of mutagens on mitotic activities in terms of reducing the number of dividing cells may have resulted in altering the genetic architecture of the plant under investigation. The cytological changes induced by the mutagens might have affected the physiological pathways that led to the alteration in various morphological and yield attributing characters. The modifications resulted would be helpful in identification and isolation of mutants with desirable characters. These mutants could be exploited for the improvement of dye yielding property of the plant. The results of the application of all the three mutagens also revealed that the genotype of the plant is highly sensitive and hence could be used for inducing the mutants in the genotype of *Rivinia humilis* L. which is one of the natural dye yielding plant that can be cultivated as cash crop, an alternative and viable source of natural dye.

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