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Research Article

EVALUATION OF INDUCED VARIABILITY IN PAPAYA (CARICA PAPAYA L.) BY PHYSICAL MUTAGENISIS

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ABSTRACT
Creation of variability is a prerequisite for crop improvement in any plant breeding programmed. Spontaneous mutations have played an important role in the improvement certain characters in some of the fruit crops. Induction of variability using physical mutagen, gamma rays has been used for long time by breeders. Induction of variability in two cultivars of papaya viz. Coorg Honey Dew and Sunrise Solo was tried using gamma rays. The seeds pretreated with 100 ppm of gibberellic acid (GA) were subjected to acute gamma irradiation with a dose rate of 10, 20, 30, 40 and 50 kR. Gamma rays significantly influenced the number of days taken for germination, germination and survival percentage, number of leaves, seedling height, total chlorophyll content, stomatal number, length and breadth in both the varieties of papaya. Irradiation of seeds with gamma rays pretreated with GA reduced chromosomal aberrations in the M1 generation. The chromosomal bridge and laggard were noticed as anaphase aberration.

Key Words: *Papaya, Mutation, Gamma Rays, Gibberellic Acid, Variability*

INTRODUCTION
Papaya (*Carica papaya* L.) is one of the important fruit crops of India being cultivated in all the tropical and sub tropical regions of the world. The technique of mutation breeding is one of the major methods of plant breeding for improvement of many crops. Creation of variability helps in the selection process. Spontaneous mutations have played an important role in the improvement of crops like grapes. Among several physical mutagenic agents, gamma irradiation is a possible tool to increase crossing over frequencies. Induction of variability using physical mutagens viz., gamma rays have been used for long time by breeders. Induction of variability in two cultivars of papaya *viz*. Coorg Honey Dew and Sunrise Solo was tried using gamma rays a physical mutagen, that produce ionizing radiations, which induce mutations.

Chromosomal aberrations produced by mutagens are the main causes of damage in mutation studies. However, experiments have revealed that great many factors modify the chromosomal damage by affecting the biological changes induced by mutagens (Gulsen, *et al*., 2007). The effect of mutagenic treatment can be modified by certain pre or post mutagenic treatment. It has been used extensively in the improvement of several crops, especially vegetative propagated species without extensive hybridization and backcrossing. Mutation breeding has led to release of more than 2,250 plant varieties in the past 70 years (Ahloowalia *et al.*, 2004). Hence, an attempt was made to study the combined effect of gamma rays irradiation and pre mutagenic treatments of seeds with gibberellic acid (GA) on germination, growth parameters and chromosomes. Gibberellic acid was used prior to mutagenic treatment with a view to enhance the efficacy of the mutagenic treatment.

MATERIALS AND METHODS
Seeds of commercial varieties of papaya *viz*. Coorg Honey Dew and Sunrise Solo were pretreated with 100 ppm of gibberellic acid (GA) for 8 hours, washed thoroughly, air dried and then subjected to acute gamma irradiation with a dose rate of 10, 20, 30, 40 and 50 kR with a check. Gamma irradiation of seeds

using Co^{60} source was done at the gamma chamber, Indian Institute of Horticultural Research, Bangalore with a dose rate 50 roentgens (rads) per minute. Each treatment was replicated three times and 50 seeds were used for each replication. Treated seeds were washed thoroughly with water and were sown in polythene bags of size 3" x 4".

Stomatal frequency was measured by the impression method. Thermocol was dissolved in xylene to get a viscous liquid and this liquid was smeared thinly on the lower surface (adaxial) of the leaf from the tip. After drying, it was peeled off and from the stomatal impressions, the number of stomata in a microscopic field (1000x) at high power were determined. Ten microscopic fields were counted for each sample. Stomatal frequency was expressed as number per cm^2 leaf area. To measure the length and breadth of stomata, ocular micrometer was calibrated using stage micrometer. Length and breadth of ten stomata in each microscopic field (150 xs) was determined and average was taken. Days taken for germination, survival per cent and number of leaves per plant**,** seedling height, chlorophyll content, mitotic chromosome number and stomatal characters were analyzed.

Chlorophyll Estimation

Fresh leaves were collected (3^{rd} and 4^{th} leaves from the top) and 100 mg of the leaf sample was placed in small beakers containing seven ml of dimethyl sulphoxide and incubated for four hours at 65^0 C in an oven. After four hours, chlorophyll extract obtained was made up to 10 ml volume using DMSO. Total chlorophyll per gram tissue was calculated by reading the optical density at 652 nm wave length and calculated by using the following formula of Hiscox and Israeistam, 1979.

$$\text{Milligram of total chlorophyll Per gram tissue} = \frac{D\ 625 \times 1000}{34.5} \times \frac{V\ (ml)}{1000 \times W\ (g)}$$

Where,

D = Optical density

V = Final volume of extract (ml)

W = Final weight of tissue extracted in gram (0.1 g)

Cytological Studies

To count the mitotic chromosome number and to observe any abnormalities, root tips were fixed in various fixatives *viz.*, cornoy's A (1 part acetic acid and 3 parts ethyl alcohol) and Carnoy's B (3 parts acetic acid, 1 part ethyl alcohol and 1 part chloroform) for 24 hours (Sharma and Sharma, 1994). Root tips were fixed at various timings starting from 8.30 am to 2.30 pm with one hour interval.

Preparation of Aceto-Orcein Stain

Distilled water (55 ml) and acetic acid (45 ml) were mixed in a conical flask and heated to boiling. Then 2 g of the dye (Orcein) was added slowly to the boiling solution. Boiling was continued till the dye was dissolved completely. The solution was cooled and filtered.

Staining Procedure

Root tips were hydrolyzed with 9 drops of the stain and 1 drop of 1N HCl taken in a watch glass at 60^0C for 5, 10 and 15 minutes which helped in maceration of the tissue as well as staining. A root tip was taken on a slide, only the portion of cell division was selected and retained. The material was squeezed in a drop of aceto orcein stain and a cover slip was placed. The tissue was warmed on a spirit lamp and tapped gently with blunt end of the needle. A uniform pressure was applied on the cover slip held under a blotter to flatten the tissue and observed under microscope. The procedure was repeated till good slide preparations were observed and photomicrographs were taken.

RESULTS AND DISCUSSION

Number of days taken for germination was significantly influenced by varied doses of gamma rays (table 1). Germination was delayed by gamma rays and the number of days taken for germination was maximum (30days) with 20 kR and minimum (16 days) in control. Gamma rays also influenced significant difference between varieties for number of days taken for germination and were 22.88 days for

Sunrise Solo and 25.22 days for Coorg Honey Dew which is probably due to the higher sensitivity of Coorg Honey Dew to gamma rays.

Table 1: Effect of gamma rays on Seed treatment

Gamma Rays	*Days Taken for Germination			**Days Taken for Germination		
Dosage	Variety			Variety		
	SS	CHD	Mean	SS	CHD	Mean
Control	12.66 (3.63)	18.66 (4.38)	15.66 (4.00)	77.55 (95.33)	75.00 (60.00)	85.16 (68.78)
10 kR	26.33 (5.18)	26.33 (5.18)	26.33 (5.18)	40.69 (42.67)	40.00 (39.13)	41.33 (39.91)
20 kR	29.66 (5.49)	30.66 (5.58)	30.16 (5.54)	28.61 (23.00)	21.00 (27.26)	22.00 (27.94)
30 kR	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)
40 kR	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)
50 kR	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)
Mean	22.88 (2.74)	25.2 (2.88)		53.67 (24.48)	45.33 (21.06)	
	V	VXD	D	V	VXD	D
SEM	0.022	0.053	0.037	0.579	1.42	1.003
CD	0.063	0.154	0.154	2.31	4.16	3.999

** SQRT Transformation SS: Sunrise Solo CHD: Coorg Honey dew:*
*** Angular Transformation*

Number of days taken for germination increased with the increase in dosage up to 20 kR. No seed germination was observed at and above 20 kR. Seed treatment with 20kR took maximum number of days (30.16) for germination, which can be attributed to the direct action of gamma rays on seeds. Interaction of varieties x doses on days taken for germination was also significant and Coorg Honey Dew took more days for germination (30.66 days) at 20 kR, which may be due to the sensitivity of this variety and direct action of gamma rays on seeds.

The varieties differed significantly and Sunrise Solo showed higher percentage (53.67) compared to Coorg Honey Dew. The increase in germination in Sunrise Solo might be due to tolerance of this variety to gamma rays. The percent germination decreased with increased dosage up to 20 kR, above 30 kR was found to be lethal. Several researchers (Ram and Srivastava, 1984 and Gulsen, *et al*., 2007) have reported that in papaya 50 kR and above were lethal. The lethality observed in this study at and above 30 kR might be due to the pretreatment of seeds with gibberellic acid, which might have increased the absorbance of gamma rays which in turn inhibited germination further. LD_{50} was found to be 9.5 kR for Sunrise Solo and 7.5 kR for Coorg Honey Dew. Interaction of varieties x doses were significant at 20kR in both the varieties.

The capability of M_1 seedlings survival up to thirtieth day is yet another factor, which brings about the efficacy of mutagens. The per cent survival of seedlings was more in Sunrise Solo (44.22) than in Coorg Honey Dew (36.11) (table 2).This shows that the efficacy and lethality varies with the variety. Survival of seedlings recorded on 30^{th} day after germination decreased with the increased dosage and seeds irradiated with 20 kR recorded lowest survival (12.99). Similar observations have been reported by several researchers in papaya and coriander. (Ram and Srivastava, 1984,; Datta, 1992 and Ahloowalia *et al.*, 2004) This may be due to decreased germination and also there is a direct relationship between dose of ionizing radiations and reduced survival percentage of seedlings which is more pronounced due to the pretreatment of seeds with gibberellic acid, which would have caused higher absorbance. The main cause

for seedling lethality appears to be failure of the roots and shoots to grow after an initial growth. LD_{50} for survival was found to be 7.0kR for Sunrise Solo and 4.5 kR Coorg Honey Dew.

Table 2: Effect of gamma rays on survival per cent and number of leaves per plant

Gamma Rays Dosage	**Survival Per Cent			*Number of Leaves		
	Variety			Variety		
	SS	CHD	Mean	SS	CHD	Mean
Control	90.33 (71.89)	70.00 (56.77)	80.16 (64.33)	9.18 (3.21)	8.07 (2.93)	8.94 (3.07)
10 kR	29.66 (32.93)	25.00 (29.93)	27.33 (31.43)	8.56 (3.01)	7.65 (2.85)	8.10 (2.93)
20 kR	20.83 (12.66)	21.40 (13.33)	21.12 (12.99)	8.32 (2.97)	6.68 (2.68)	7.50 (2.82)
30 kR	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)
40 kR	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)
50 kR	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)
Mean	44.22 (20.94)	36.11 (18.02)		8.89 (1.88)	7.47 (1.76)	
	V	VXD	D	V	VXD	D
SEM	0.007	0.017	0.012	0.012	0.029	0.021
CD	0.020	0.049	0.035	0.035	0.086	0.061

** SQRT Transformation* *** Angular Transformation*

The number of leaves showed significant difference between the varieties and was more in Sunrise Solo (8.89) compared to Coorg Honey Dew. Varieties x dosage did influence the number of leaves and was minimum in Coorg Honey Dew at 20 kR (6.68).Varietal differences and reduction in the number of nodes by inhibitory effect of gamma rays together is responsible for the difference in number of leaves in interaction effect.

Significant difference in seedling height 15 and 30 days after germination was observed between varieties with Sunrise Solo recording maximum seedling height (6.81 and 11.22 cm respectively) compared to Coorg Honey Dew. Seedling height decreased with the increased dosage Varieties x dosage also showed significant result at 15 and 30 days after germination with respect to seedling height and Coorg Honey Dew recorded maximum seedling height of 5.50 and 9.58 cm respectively at 20 kR (table 3).

Table 3: Effect of Seed treatment with gamma rays on seedling height in Sunrise Solo and Coorg Honey Dew

Gamma Rays Dosage	*15 Days After Germination			*30 Days After Germination		
	Variety			Variety		
	SS	CHD	Mean	SS	CHD	Mean
Control	7.80 (2.88)	6.50 (2.65)	7.15 (2.76)	12.63 (3.62)	11.23 (3.42)	11.93 (3.52)
10 kR	6.76 (2.69)	6.44 (2.63)	6.60 (2.66)	11.73 (3.49)	10.73 (3.35)	11.23 (3.42)
20 kR	5.86 (2.52)	5.50 (2.45)	5.68 (2.48)	9.31(3.13)	3.17 (9.31)	9.44 (3.15)
30 kR	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)
40 kR	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)
50 kR	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0(0.71)	0.0 (0.71)
Mean	6.81(1.70)	6.15 (1.64)		11.22 (2.01)	10.51 (2.01)	
	V	VXD	D	V	VXD	D
SEM	0.335	0.200	0.059	0.017	0.041	0.029
CD	1.334	3.268	2.311	0.049	0.121	0.085

** SQRT Transformation*

Similar findings were reported by Crossman and Craig (1982 and Ahloowalia *et al.*, 2004). Reduction in seedling height in M1 generation is due to the changes in the levels of auxins and abscicic acid content due to physiological and biochemical disturbances or changes in enzyme activity or radiation might have affected the meristem interfering with cell progression as reported earlier (Raghuvanshi and Singh, 1979). Total chlorophyll Content did not differ significantly between the varieties (table 4). However total chlorophyll content decreased with the increase in dosage up to 20 kR which recorded the lowest content (1.54 mg g^{-1} tissue). Inhibition of chlorophyll should have been caused by inhibition of DNA, RNA and protein synthesis. Number of stomata differed significantly between the varieties with Sunrise Solo having more number of stomata (17479.35 cm^{-2} compared to Coorg Honey Dew (14743.58cm^{-2}) (table 4).

Table 4: Effect of Seed treatment with gamma rays on total chlorophyll content and Stomata number in Sunrise Solo and Coorg Honey Dew

Gamma Rays Dosage	*Total Chlorophyll Content			*Number of Stomata		
	Variety			Variety		
	SS	**CHD**	**Mean**	**SS**	**CHD**	**Mean**
Control	2.12 (1.62)	2.19 (1.64)	2.15 (1.63)	19333.3 (138.98)	18871.78 (137.36)	19102 (138.17)
10 kR	1.77 (1.51)	1.68 (1.48)	1.72 (1.49)	16822.50 (129.69)	13282.05 (115.12)	15052.27 (122.41)
20 kR	1.51(1.42)	1.58 (1.44)	1.54(1.43)	16282.27 (127.31)	12076 (109.87)	14179.59 (118.59)
30 kR	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)
40 kR	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)
50 kR	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)
Mean	1.80 (1.11)	1.82 (1.11)		17479.35 (66.35)	14743.58 (60.75)	
	V	**VXD**	**D**	**V**	**VXD**	**D**
SEM	.005	0.012	0.009	0.964	2.362	1.670
CD	NS	NS	0.036	2.814	6.892	4.873

** SQRT Transformation*

The number deceased with the increase in gamma rays dosage and the seeds irradiated with 20 kR had the least number of stomata (14743.58 cm^{-2}). Datta and Banerji (1990) in bougainvillea also reported the similar results. The interaction of varieties x dosage showed significant difference with Coorg Honey Dew recording least number of stomata (12076.92 cm-$^{2)}$ at 20 kR.

Significant differences were observed only with the dosage for stomatal length and breadth and the maximum stomatal length (16.08 u) and breadth (8.54 u) was recorded at 20 kR. This finding is contradictory to the inference made by Datta and Banerji (1990) who noticed reduced stomatal length breadth in Bogounvillea after irradtion with gamma rays. The increased stomatal length breadth in this study can be correlated to Gibberlic acid pretreatment given before mutagenic treatment which might have modified the effect.

Gamma rays irradiation of papaya seeds pretreated with GA reduced chromosomal aberrations in the M_1 generation. The anaphase aberration noticed was chromosomal bridge and laggard (table 5).

Reduced chromosomal aberrations in gamma rays irradiation treatment might have been due to GA pretreatment which is an effective modifier of chromosomal damage. GA probably reduces the chromosome aberrations by activating the inactivated SH groups containing oxidative enzymes and

thereby increasing overall cellular oxidation which releases high energy to the chromosome – repairing system (Dina, 2001).

Table 5: Effect of Seed treatment with gamma rays on length and breadth of Stomata in Sunrise Solo and Coorg Honey Dew

Gamma Rays Dosage	*Stomatal Length(u)			*Stomatal Breadth(u)		
	Variety			Variety		
	SS	CHD	Mean	SS	CHD	Mean
Control	11.25 (3.43)	9.83 (4.21)	10.54 (3.32)	5.81(2.51)	5.71(2.49)	5.76 (2.50)
10 kR	15.96 (4.06)	14.57 (3.88)	15.26 (3.97)	7.20 (2.77)	8.33 (2.97)	7.76 (2.87)
20 kR	16.33 (4.10)	15.83 (4.04)	16.08 (4.07)	8.46 (2.99)	8.63 (3.02)	8.54 (3.00)
30 kR	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)
40 kR	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)
50 kR	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)	0.0 (0.71)
Mean	14.51(2.28)	13.41(2.21)		7.16 (1.73)	7.56 (1.77)	
	V	VXD	D	V	VXD	D
SEM	0.012	0.030	0.022	0.001	0.004	0.002
CD	NS	NS	0.063	NS	NS	0.075

** SQRT Transformation*

Greater emphasis is to be given to the use of combined treatment of physical mutagen and growth regulators. The experiment clearly shows that radiation induced genetic damage and mutation frequencies can be modified and influenced by treatment of seeds with growth regulators before or after irradiation. Growth regulators are not only mutagenic by themselves but also affect mutation in specific ways when applied in combination with radiation. The combined use of this can help the breeder in creating better variability thereby better scope for selection.

REFERENCES

Ahloowalia BS, Maluszynski M and Nichterlein K (2004). Global impact of mutation-derived varieties. *Euphytica* **135**(2) 187-204.

Crossman HH and Craig R (1982). The effect of gamma irradiation of seeds on germination and plant morphology of Pelargonium x hortum. *Journal of American Sociology Horticulture Science* **107**(10) 72-75.

Datta SK (1992). Assessment of single and combined x-ray and colchicines treatment on *Trichosanthes anguina* L. *Journal of Nuclear Agriculture Biology* **21**(4) 293-298.

Datta SK and Banerji BK (1990). Los Banos Variegata-new double bracted chlorophyll variegated bougainvillea induced by gamma rays. *Journal of Nuclear Agriculture Biology* **19**(2) 134-136.

Dina Z and Abdul Kader (2001). Draught and Gibberellic Acid dependent oxidative stress effect on antioxidant defence system in two lettuce cultivars. *Pakistan Journal of Biological Sci*ence **4**(9) 1143-2001.

Gulsen O, Uzun A, Pala H, Canilhos E and Kafa G (2007). Development of seedless and Mal seco tolerant mutant lemons through bud wood irradiation. *Science Horticulture* **112** 184-190.

Raghuvanshi SS and Singh AK (1979). Gamma-Ray Induced Mutations in Diploid and Autotetraploid Perennial *Portulaca Grandiflora*, Hook. *Indian Journal of Horticulture* **36**(1) 84-87**.**

Ram M and Shrivastava S (1984). Mutagenesis in papaya. *National Seminar on Papaya and Papain Production* 26-27.

Sharma AK and Sharma A (1994). Chromosome Techniques: A manual. *Harwood Academic Publishers* 1-368.