IRRADIATION INDUCED MUTAGENESIS IN SUGARCANE

Thesis

Submitted to the Punjab Agricultural University in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE
in
PLANT BREEDING AND GENETICS
(Minor Subject: Plant Pathology)

By

Mandeep Kaur
(L-2011-A-74-M)

Department of Plant Breeding and Genetics
College of Agriculture
© PUNJAB AGRICULTURAL UNIVERSITY
LUDHIANA-141004

2014

CERTIFICATE I

This is to certify that the thesis entitled, **"Irradiation induced mutagenesis in sugarcane"** submitted for the degree of **M.Sc.** in the subject of **Plant Breeding and Genetics** (Minor subject: **Plant Pathology**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Mandeep Kaur (L-2011-A-74-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

_____

Major Advisor
(Dr. K.S. Thind)
Director
PAU Regional Research Station
Kapurthala.

CERTIFICATE II

This is to certify that the thesis entitled, **"Irradiation induced mutagenesis in sugarcane"** submitted by **Mandeep Kaur (L-2011-A-74-M)** to the Punjab Agricultural University, Ludhiana, in partial fulfilment of the requirements for the degree of **M.Sc.** in the subject of **Plant Breeding and Genetics** (Minor Subject: **Plant Pathology**) has been approved by the Student's Advisory Committee along with the Head of Department after an oral examination of the same.

_____
Major Advisor
(Dr. K.S. Thind)

_____
External Examiner

_____
Head of the Department
(Dr. R.K. Gumber)

_____
Dean, Postgraduate Studies
(Dr. Gursharan Singh)

ACKNOWLEDGEMENTS

*First of all, I bow my head to the **ALMIGHTY** for bestowing a healthy environment throughout my academic and research work and blessing me with skills so as to be all what I am today.*

No one can ever make any achievement alone. The successful completion of this manuscript was made possible through the invaluable contribution of a number of people.

*I wish to record my heartful gratitude to my Major Advisor **Dr. K.S. Thind**, Director PAU, Regional Research Station Kapurthala, for his constructive guidance, constant motivation and polite dealing during my research as well as in preparation of this manuscript. I would also like to thank him for being an open person to ideas and giving me freedom to work in the lab in my own way.*

*I sincerely acknowledge to the members of my advisory committee Dr. R S Gill (Breeder), **Dr. Vipen Sharma** (Plant Pathologist) and **Dr. M S Grewal** (Nominee Dean PGS), for their cooperation at every moment when required.*

*I am highly indebted to **Dr. Gulzar Sanghera** (Breeder Sugarcane), Department of Plant breeding & Genetics for guiding me throughout my research work, providing me all the necessary facilities and infrastructure for accomplishing the research work and critically editing the manuscript. My sincere thanks to supporting staff members of Regional Research Station Kapurthala, field and office staff for their help during my research work.*

*A formal acknowledgement is inadequate to convey my gratitude to my **family**, my loving parents Sh. Jai Bhagwan Dass and Smt. Kamla Devi, my brother, bhabi and Sister for their love, affection, belief, blessings, moral and economical support which enabled me to put this tireless venture to a fruitful result. Without them I could not have made it here.*

Words never can but for the pleasure of it, I express my thanks to my friends Jagdeep, Navneet, Ruqiya, Priti, Sukhi, Puneet, Arpan, Rohini, Gomti, Govind, Harry and Inderjit for their support, memorable company, ever willing help, and for providing me few moments of refreshment in between the exhausting hours to shed the workload which kept me energetic during this period and made my stay at the university memorable. Cheers to Gomti for being a supportive person with whom I discussed my problems related to research.

Memory and words may fail to acknowledge somebody but the heart can't. Heart-felt thanks to all for providing me even a minuscule of help during this period of investigation.

(Mandeep Kaur)

Title of the Thesis	:	Irradiation induced mutagenesis in sugarcane
Name of the Student and Admission No.	:	Mandeep Kaur (L-2011-A-74-M)
Major Subject	:	Plant Breeding and Genetics
Minor Subject	:	Plant Pathology
Name and Designation of Major Advisor	:	Dr. K.S. Thind Director, PAU Regional Research Station Kapurthala
Degree to be Awarded	:	M.Sc.
Year of Award of Degree	:	2014
Total Pages in Thesis	:	47 + Vita
Name of University	:	Punjab Agricultural University Ludhiana-141 004, Punjab (India)

ABSTRACT

A study on irradiation induced mutagenesis in sugarcane was undertaken to investigate genetic variability for economic traits and red rot disease. Twelve hundred single bud setts of cv. Co J 64 were irradiated with gamma rays at 0, 10, 20, 25 and 30 Grays (Gy). The experimental material of M_1V_1 generation was planted during spring 2012 and second generation (M_1V_2) during 2013. Data was recorded on ten economic and quality traits in addition to reaction to red rot disease. Mutagenic treatments generated significant variation for germination, number of tillers/clump, number of millable canes/clump, cane height, HR brix, leaf length and single cane weight in both M_1V_1 and M_1V_2 generations indicating the potential of mutagenic treatments for creating genetic variability for different traits in sugarcane. Average cane height among different treatments ranged from 166.60 to 185.80 cm with a mean of 171.2 cm. Mean leaf length for different treatments varied from 106.30 to 114.80 cm with an average of 111.40 cm; maximum of 114.80 cm being recorded through 10 Gy treatment followed by control**.** Cane weight an important trait in sugar cane breeding programme ranged from 720.00 to 961.66 g. Maximum single cane weight was recorded for control followed by 10 Gy and 20 Gy treatments. The higher doses had a retarding effect on this trait. Artificial evaluation of irradiated subclones against two red rot pathotypes Cf 08 and Cf 09 using plug method revealed moderate resistance for all the mutagenic treatments. It is inferred that genetic variability could be induced in sugarcane using gamma rays for traits like germination, cane height, leaf length, HR brix, single cane weight and to red rot tolerance. The lower doses of gamma ray (10 and 20 Gy) were more effective to induce variation while higher doses have detrimental effects. The sub clones need to be further evaluated in M_1V_3 for their consistency in trait manifestation and future use in breeding programme.

Signature of the Major Advisor **Signature of the student**

ਖੋਜ ਪ੍ਰਬੰਧ ਦਾ ਸਿਰਲੇਖ	:	ਗੰਨੇ ਵਿੱਚ ਜਿਣਸੀ ਤਬਦੀਲੀਆਂ ਲਈ ਤੇਜ ਰੌਣਕੀ ਉਪਚਾਰ
ਵਿਦਿਆਰਥੀ ਦਾ ਨਾਂ ਅਤੇ ਦਾਖ਼ਲਾ ਨੰਬਰ	:	ਮਨਦੀਪ ਕੌਰ ਐਲ-2011-ਏ-74-ਐਮ
ਮੁੱਖ ਵਿਸ਼ਾ	:	ਪਲਾਂਟ ਬ੍ਰੀਡਿੰਗ ਅਤੇ ਜਨੇਟਿਕਸ
ਸਹਿਯੋਗੀ ਵਿਸ਼ਾ	:	ਪੌਦਾ ਰੋਗ ਵਿਗਿਆਨ
ਮੁੱਖ ਸਲਾਹਕਾਰ ਦਾ ਨਾਂ ਅਤੇ ਅਹੁਦਾ	:	ਡਾ. ਕੇ.ਐੱਸ. ਥਿੰਦ ਡਾਇਰੈਕਟਰ, ਪੀ.ਏ.ਯੂ. ਰੀਜ਼ਨਲ ਰਿਸਰਚ ਸਟੇਸ਼ਨ ਕਪੂਰਥਲਾ
ਹਾਸਲ ਹੋਣ ਵਾਲੀ ਡਿਗਰੀ	:	ਐਮ.ਐਸ.ਸੀ.
ਡਿਗਰੀ ਮਿਲਣ ਦਾ ਸਾਲ	:	2014
ਖੋਜ ਪ੍ਰਬੰਧ ਵਿੱਚ ਕੁੱਲ ਪੰਨੇ	:	47 + ਵੀਟਾ
ਯੂਨੀਵਰਸਿਟੀ ਦਾ ਨਾਮ	:	ਪੰਜਾਬ ਖੇਤੀਬਾੜੀ ਯੂਨੀਵਰਸਿਟੀ, ਲੁਧਿਆਣਾ-141004 ਪੰਜਾਬ, ਭਾਰਤ ।

ਸਾਰ ਅੰਸ਼

ਗੰਨੇ ਵਿੱਚ ਵਿਕਿਰਣ ਪ੍ਰੇਰਿਤ mutagenesis ਉੱਤੇ ਇੱਕ ਖੋਜ, ਆਰਥਕ ਲੱਛਣ ਅਤੇ ਰਤਾ ਰੋਗ ਲਈ ਆਨੁਵੰਸ਼ਿਕ ਪਰਿਵਰਤਨਸ਼ੀਲਤਾ ਦੀ ਜਾਂਚ ਲਈ ਕੀਤੀ ਗਈ । CoJ 64 ਕਿਸਮ ਦੇ ਇੱਕ ਹਜ਼ਾਰ ਦੋ ਸੌ, ਇੱਕ ਅੱਖ ਵਾਲੇ ਬਰੋਟੇਆਂ ਦਾ 0, 10, 20, 25 ਅਤੇ 30 Gy ਤਾਕਤ ਵਾਲੀ ਗਾਮਾ ਕਿਰਨਾਂ ਦੇ ਨਾਲ ਵਿਕਿਰਣ ਕੀਤਾ ਗਿਆ । M_1V_1 ਪੀੜ੍ਹੀ ਦੇ ਪ੍ਰਯੋਗਾਤਮਕ ਪੌਦਿਆਂ ਦੀ 2012 ਦੌਰਾਨ ਅਤੇ ਦੂਜੀ ਪੀੜ੍ਹੀ (M_1V_2) ਦੀ 2013 ਦੇ ਬਸੰਤ ਦੌਰਾਨ ਬੁਆਈ ਕੀਤੀ ਗਈ । ਦਸ ਆਰਥਕ ਅਤੇ ਗੁਣਵੱਤਾ ਲੱਛਣਾਂ ਅਤੇ ਰੱਤਾ ਰੋਗ ਦੀ ਪ੍ਰਤੀਕਿਰਿਆ ਦਾ ਡਾਟਾ ਲੈ ਕੇ ਵਿਸ਼ਲੇਸਣ ਕੀਤਾ ਗਿਆ । Mutagenic ਉਪਚਾਰ ਤੋਂ ਪੈਦਾ ਹੋਈ ਆਨੁਵੰਸ਼ਿਕ ਪਰਿਵਰਤਨਸ਼ੀਲਤਾ ਦੇ ਸੰਭਾਵਿਕ ਸੰਕੇਤ M_1V_1 ਅਤੇ M_1V_2 ਪੀੜੀਆਂ ਵਿੱਚ ਉਗਣ ਸ਼ਕਤੀ, ਟਿਲਰ ਪ੍ਰਤੀ ਝੁਰਮੁਟ ਦੀ ਗਿਣਤੀ, ਮਿੱਲ ਦੀ ਵਰਤੋਂ ਯੋਗ ਗੰਨੇ ਪ੍ਰਤੀ ਝੁਰਮਟ, ਗੰਨੇ ਦੀ ਲੰਬਾਈ, HR Brix, ਪੱਤੀ ਦੀ ਲੰਬਾਈ ਅਤੇ ਚੌੜਾਈ, ਅਤੇ ਏਕਲ ਗੰਨੇ ਦਾ ਭਾਰ ਆਦਿ ਲੱਛਣਾ ਲਈ ਮਹੱਤਵਪੂਰਨ ਪਰਿਵਰਤਨ ਵੇਖੇ ਗਏ । ਪਰਿਵਰਤਿਤ ਗੰਨੇ ਵਿੱਚ ਗੰਨੇ ਦੀ ਲੰਬਾਈ 166.60 - 185.80 ਸੈ.ਮੀ. ਵੇਖੀ ਗਈ ਅਤੇ ਔਸਤ ਲੰਬਾਈ 171.2 ਸੈ.ਮੀ. ਸੀ । ਪੱਤੀ ਦੀ ਲੰਬਾਈ 106.30 - 114.80 ਸੈ.ਮੀ. ਦਰਜ ਕੀਤੀ ਗਈ ਅਤੇ ਔਸਤ ਲੰਬਾਈ 111.4 ਸੈ.ਮੀ. ਰਹੀ । ਅਧਿਕਤਮ ਪੱਤੀ ਦੀ ਲੰਬਾਈ 114.80 ਸੈ.ਮੀ., 10 Gy ਵਿਕਰਣ ਦੇ ਮਾਧਿਅਮ ਵੱਲੋਂ ਪ੍ਰਾਪਤ ਹੋਈ । ਏਕਲ ਗੰਨਾ ਭਾਰ, ਗੰਨਾ ਪ੍ਰਜਨਨ ਪ੍ਰੋਗਰਾਮ ਵਿੱਚ ਇੱਕ ਮਹੱਤਵਪੂਰਨ ਵਿਸ਼ੇਸ਼ਤਾ ਰੱਖਦਾ ਹੈ । ਇਸ ਖੋਜ ਦੌਰਾਨ ਏਕਲ ਗੰਨਾ ਭਾਰ 720.00 - 961.66 ਗ੍ਰਾਮ ਪ੍ਰਾਪਤ ਹੋਇਆ । ਅਧਿਕਤਮ ਏਕਲ ਗੰਨਾ ਭਾਰ 10 Gy ਅਤੇ 20 Gy ਵਿਕਰਣ ਦੁਆਰਾ ਦਰਜ ਕੀਤਾ ਗਿਆ । ਵਿਕਿਰਣ ਦੀ ਵੱਧਦੀ ਮਾਤਰਾ ਦਾ ਇਸ ਵਿਸ਼ੇਸ਼ਤਾ ਉੱਤੇ ਮਾੜਾ ਪ੍ਰਭਾਵ ਪਿਆ । ਦੂਜੀ ਪੀੜ੍ਹੀ (M_1V_2) ਦੇ ਵਿਕਿਰਣਿਤ subclones ਨੂੰ ਪੱਲਗ ਪੱਧਰੀ ਨਾਲ ਰੱਤਾ ਰੋਗ (Cf 08 ਅਤੇ Cf 09) pathotypes ਦੇ ਟੀਕੇ ਲਗਾਏ ਗਏ ਅਤੇ ਇਹਨਾਂ ਵਿੱਚ ਬਦਲਾਓ ਵੇਖਿਆ ਗਿਆ । ਰੱਤਾ ਰੋਗ ਲਈ ਕੁਝ subclones ਵਿੱਚ ਮੱਧ ਪ੍ਰਤੀਰੋਧ ਦਰਜ ਕੀਤਾ ਗਿਆ । ਭਾਵ ਇਹ ਕਿ ਆਨੁਵੰਸ਼ਿਕ ਪਰਿਵਰਤਨਸ਼ੀਲਤਾ ਅੰਕੁਰਣ, ਗੰਨੇ ਦੀ ਲੰਬਾਈ, HR Brix, ਏਕਲ ਗੰਨਾ ਭਾਰ ਅਤੇ ਰੱਤਾ ਰੋਗ ਸਹਿਨਸ਼ੀਲਤਾ ਲਈ ਗਾਮਾ ਕਿਰਨਾਂ ਦੀ ਵਰਤੋਂ ਕਰਨ ਨਾਲ ਪਰਿਵਰਤਨ ਪ੍ਰੇਰਿਤ ਕੀਤਾ ਜਾ ਸਕਦਾ ਹੈ । ਗਾਮਾ ਕਿਰਨਾਂ 10 ਅਤੇ 20 Gy ਦੀ ਮਿਕਦਾਰ ਵਿੱਚ ਭਿੰਨਤਾ ਪ੍ਰੇਰਿਤ ਕਰਣ ਲਈ ਜ਼ਿਆਦਾ ਪਰਭਾਵੀ ਰਹੀਆਂ ਜਦੋਂ ਕਿ ਜਿਆਦਾ ਮਾਤਰਾ ਨੁਕਸਾਨਦਾਇਕ ਸਿੱਧ ਹੋਈ । M_1V_3 ਦੇ ਕਲੋਨਸ ਵਿੱਚ ਵੀ ਵਿਸ਼ੇਸ਼ਤਾ ਕਰਨ ਅਤੇ ਪ੍ਰਜਨਨ ਪ੍ਰੋਗਰਾਮ ਵਿੱਚ ਭਵਿੱਖ ਵਿੱਚ ਵਰਤੋਂ ਲਈ ਲੇਖਾ ਜੋਖਾ ਕੀਤੇ ਜਾਣ ਦੀ ਸਿਫਾਰਸ਼ ਚਾਹੀਦਾ ਕੀਤੀ ਜਾਂਦੀ ਹੈ ।

ਮੁੱਖ ਸ਼ਬਦ:- Mutagenesis, ਰੱਤਾਰੋਗ, ਜਿਣਸੀ ਤਬਦੀਲੀ, ਗੰਨਾ, ਗਾਮਾ ਕਿਰਨਾਂ ।

ਮੁੱਖ ਸਲਾਹਕਾਰ ਦੇ ਹਸਤਾਖਰ | **ਵਿੱਦਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ**

CONTENTS

CHAPTER	TITLE	PAGE NO.
I	INTRODUCTION	1-3
II	REVIEW OF LITERATURE	4-16
III	MATERIAL AND METHODS	17-22
IV	RESULTS AND DISCUSSION	23-35
V	SUMMARY	36-38
	REFERENCES	39-47
	VITA	

LIST OF TABLES

TABLE NO.	TITLE	PAGE NO.
1	Different irradiation treatment combination used in study	17
2	Analysis of variance for different traits in sugarcane (M_1V_1) sub clones of Co J 64 at USF, Ladhowal during spring season 2012	23
3	Analysis of variance for different traits in sugarcane (M_1V_2) sub clones of Co J 64 at RRS, Kapurthala grown during spring season 2013	24
4	Performance of important characteristics of M_1V_1 irradiated sub-clones of Co J 64 grown during spring 2012	25
5	Performance of important characteristics of M_1V_2 irradiated sub-clones of Co J 64 grown during spring 2013	29
6	Disease reaction of irradiated sub clones of Co J64 against two red rot pathotype in M_1V_2 generation	35

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE NO.
1	Percent clumps in M_1V_2 generation showing variation in number of tillers at different doses γ-irradiation	31
2	Percent clumps in M_1V_2 generation showing variation in number of millable canes at different doses γ-irradiation	31
3	Variability for cane height in M_1V_2 generation at different doses of γ-irradiation	32
4	Variability for cane girth in M_1V_2 generation at different doses of γ-irradiation`	33
5	Variability for HR Brix in M_1V_2 generation at different doses of γ-irradiation	33
6	Variability for single cane weight in M_1V_2 generation at different doses of γ-irradiation	34

LIST OF PLATES

PLATE NO.	TITLE
1	Gamma Chamber
2	Refractometer
3	Inoculation under field conditions using plug method
4	Irradiated sub-clones (M_1V_2 generation) showing differential response to red rot disease (artificial inoculation)

CHAPTER - I

INTRODUCTION

Sugarcane *(Saccharum* spp. complex) is an important agro-industrial crop that accounts for nearly two-thirds of world sugar production. It is chiefly grown in the tropical and subtropical regions. Commercial production of sugar from sugarcane began in India and China approximately 2500 years ago and spread to Western Europe in the eighteenth century (James 2004). Today, in addition to sugar production, sugarcane and its by-products are used as raw materials in the production of fuel, chemicals, bio-fertilisers, paper and pulp etc with the possibility of more products being produced through bio-refining (Arruda 2011). India is the largest producer next only to Brazil and is also a major consumer of sugar, with only 3-5% of the production being exported. With the growing emphasis on production of bio-ethanol and its blending with fuel, importance of sugarcane would grow in the coming years, as it produces about 4000-6000 li/ha of ethanol. Besides sugar and ethanol, nearly 30 by-products can be produced from sugarcane for value addition.

Sugarcane is a highly heterozygous polyploid species (2n=80 to 205). Modern sugarcane varieties are complex hybrids arising primarily from *S. officinarum, S barberi, S. sinese* hybridized with *S. spontaneum.* Natural or induced genetic diversity is a vital component of a plant breeding program. In sugarcane, molecular diversity studies have revealed that the modern commercial cultivars have been developed from a limited genetic base and were derived from only 20 *S. officinarum* clones and less than ten *S. spontaneum* derivatives (Sreenivasan *et al* 1987).

Development of a sugarcane variety with stable performance has been a challenging task for sugarcane breeders. Sugarcane produces flowers in specific environmental niches i.e. in tropical regions. Due to its heterozygous and polyploid nature, the flowering behaviour of sugarcane genotypes poses many challenges to sugarcane breeders *viz.* (i) poor fertility, (ii) non-synchrony, (iii) non-flowering of specific genotypes etc. Furthermore, any genotype developed may not be perfect in all aspects. Therefore, there is a need to diversify the genetic base of this crop.

Successful use of plant breeding for improving crops requires the existence of genetic variation for useful traits but the desired variation is often lacking. However, radiation can be used to induce mutations and thereby generate genetic variation from which desired mutants may be selected. Mutation is a sudden heritable change in organism generally the structural change in gene. The term mutation breeding was first coined by Freisleben and Lein (1944) referring to deliberate induction and development of mutant lines of crop improvement. Mutation breeding is one of the conventional breeding methods in plant breeding. It is relevant with various fields like, morphology, cytogenetics, biotechnology and molecular

biology etc. It's produced by change in the base sequence of genes and it can be induced either spontaneously or artificially both in seed and Vegetative Propagated crops. Induced mutations offer the possibility of generating desired attributes that either cannot be expressed in nature or have been lost during evolution (Brunner 1995). Mutation breeding has made significant contributions by way of development of more than 3100 mutant cultivated varieties in about 190 plant species. Mutations have also been found to be effective in improving one or few traits in an otherwise agronomically adapted variety.

Direct use of mutations is a very valuable supplementary approach of plant breeding and the main advantage of mutation induction in vegetatively propagated plants is the ability to change one or few characters of an outstanding cultivar without altering the remaining genotype.

Mutations may be recessive or dominant but the former is more common although the cannot express phenotypically unless two recessive genes come together as homozygotes (Smith 1958, Ehrenberg 1960, Hrishi and Marimuthamal 1968). This expression requires one or more generations of recombination of two or more similar recessives to affect the phenotypic appearance in the population. The screening and selection of mutants have been reported to be preferable from second irradiation generation because in case of recessives both loci are rarely mutated, and therefore segregation must be permitted so that mutants can be isolated in homozygous condition (Favret 1960).

In sugarcane, most recessive mutants have been selected for the improvement of many characters. Several breeders have reported the successful use of mutations for developing genotypes resistant to diseases in sugarcane (Jagathesan *et al* 1974; and Srivastava *et al* 1986), sugarcane mosaic virus (Breanx 1975, Dermodjo 1977, Siddiqui and Javed 1982). Induced mutations in sugarcane have been reported for desirable traits and **to** induce red rot resistant mutants of sugarcane variety Co S 687 using gamma rays and chemical mutagens (Siddiqui and Javed 1982).

It was observed that a 4 kR dose of ionizing radiations was fatal for cane cuttings (Tysdal 1956), but on other hand (Panje and Prasad 1959) reported that 7.2 kR had little effect with 50% mortality at 14.4 kR. Vijailakshmi & Rao (1960) and Siddiqui & Javed (1982) reported that 3 kR was the safe dose to induce mutations in sugarcane. Price and Warner (1959) outlined the possible use of mutations for sugarcane improvement. To overcome the problems of generating variability, augmenting the conventional breeding like induction of mutants was seen as a potent tool for sugarcane breeder (Patade and Suprasanna 2008).

In this study, sugarcane variety Co J 64 was selected because it is an early maturing variety developed from the cross (Co 976 x Co 617). It covered nearly 80% sugarcane area in Punjab during 1980's. It is short duration (maturing in 8 months), high sugared, high yielding but susceptible to red rot disease caused by *Colletotrichum falcatum* went which poses a

challenge to sugarcane cultivation in many parts. It has medium thick greenish yellow canes with barrel shaped internodes. Despite extensive breeding efforts, till date no genotype has surpassed the early maturity and high quality characteristics of Co J 64, and it is still the quality check for the North West zone of the country. Efforts to develop red rot resistance through conventional breeding have not been fruitful. So, efforts have been made in present study entitled ‘ Irradiation induced mutagenesis in sugarcane’ to generate variability for yield and quality traits in addition to induce red rot resistance in sugarcane variety Co J 64 using gamma rays with following objectives:

Objectives

- Use of gamma irradiations on somatic tissue (buds) of sugarcane to generate variability.
- Estimation of genetic variability generated in the mutagenic population for different agronomic and quality traits and for reaction to red rot disease.

CHAPTER – II

REVIEW OF LITERATURE

The use of physical mutagens in mutation breeding dates back to the early 20th century with the use of X-rays and later, gamma and neutron radiation (Novak and Brunner 1992). They have been used in mutation breeding of many crops including sugarcane. Mutation efficiency of physical mutagenic agents depends on the dose, dose rate, dose distribution and exposure time (Brunner 1995, Suprasanna *et al* 2009). The establishment of these parameters relies upon radiation type, radiation facilities and the type of material to be exposed to radiation (Brunner 1995). X-rays and gamma rays can penetrate deep into the tissue due to limited scattering and concentration of the ion beam on the plant tissue leading to high mutation frequency compared with UV-light and neutron radiation (Suprasanna *et al* 2009). Physical mutagens are less hazardous and are easier to handle compared to chemical mutagens (Suprasanna *et al* 2009), but are relatively expensive due to the equipment required (Poehlman and Sleper 1995).

Physical mutagens both ionizing and non-ionizing radiations are widely used to induce mutations in different kinds of plant parts. Ionising radiations generally used are X – rays and gamma rays. Mutation induction with radiation was the most frequently used method to develop mutant varieties and gamma rays were employed to develop 64 per cent of the radiation induced mutant varieties (Ahloowalia *et al* 2004). In India also, out of 313 mutant varieties developed so far, 169 mutant varieties were obtained by using gamma rays (Chopra 2005). Gamma rays have been commonly used for sugarcane mutagenesis.

Mutation studies in sugarcane

Rao (1954) obtained an increase in colour range of stripes and differences in flowering propensity following exposure of buds of a striped variety of sugarcane to X-ray.

Out of 164 *In vitro* selected resistant lines, after two years field trials, only 8 were found to be resistant against red rot disease according to red rot disease rating scales as proposed by Srinivasan and Bhat (1961).

Plants obtained from vegetative buds of sugarcane variety Co 449 were exposed to 500 r, 1000 r, 2000 r, 3000 r, 4000 r, 5000 r and 10000 r of gamma radiation from a ^{60}Co source were inoculated with the D strain. One plant each from the vegetative progeny of buds subjected to dosages of 500 r and 3000 r were found to be resistant while all the others (an average of about 1500 for each dosage) were susceptible. The mutant clones were morphologically indistinguishable from the original variety (Rao *et al* 1966).

Many factors are involved in radio sensitivity and the best practice is empirical one i.e. the starting material should be first exposed to a range of doses, centered around the

optimum one, either that found in literature or a calculated optimum (Sparrow *et al* 1967).

Hrishi and Marimuthamal (1968) treated setts of sugarcane cv Co 419 with five chemical mutagens. Mutations for shortened internodes and glabrous leaf sheath were induced. Some tissue damage was observed, like dissected lamina.

Malhotra and Vijaybhanu (1969) found stripped chimera in the sugarcane variety Bo 14 and a chimera with copious ivory markings in Bo17. Other morphological characters and juice quality of both these clones resembled with those of normal plants. Chimera of Bo 32 showed a marked reduction in size and brix values and were susceptible to *P tucumanensis.* Jagathesan and Srinivasan (1970) observed growth stimulation by applying low doses of X-rays in main shoot in some of the varieties.

Mustafakhan and Zakir (1970) observed improvement in quality of sugarcane juice in X1 and X2 generations with a dose of 7.5 krad and in cane weight in the X1 generation with a dose of 4 krad. Increased sugar content was obtained by Shankaranarayan and Babu (1970) by applying 1500 r to 2000 r dose. Some promising mutants of the varieties Co 527 and Co 663 have been obtained by Haq *et al* (1970) following gamma radiation.

Cane setts of variety Co 997 were irradiated with gamma rays at dose rate of 2000 rad. This resulted in the production of a mutant highly resistant to red rot. The mutant was similar to the original non-irradiated stock in all other respects (Singh 1970). Four varieties of sugarcane were irradiated with Gamma rays and X-rays at doses 1, 2, 3, 4 and 5 kr. Ml, M2 and M3 generations were studied. A useful glabrous leaf sheath mutant, breeding true in the M3 generation was isolated from cvs Co 419 and Co 527 (Jagathesan and Ratnam 1970). Irradiation and chemical mutagenic treatment was applied in the sugarcane varieties Co 997, Co449, Co527 and Co312. By applying this treatment induced mutants with *P tucumanensis* resistance have been obtained (Shah 1972). Nair (1973) was able to isolate Co 997-24-1 red rot resistant mutant which was similar to Co 997 a susceptible variety for yield of cane and sugar and for sucrose content of the juice.

Rao (1974) described the breeding behavior of first generation mutants from bud setts of Co 419 that had been treated with gamma rays. Bari (1974) exposed buds of four high yielding sugarcane varieties BL 4, BL 19, CoL 54 and L 116 were exposed to 2, 3 and 4 KR dose of gamma rays. Some mutants were obtained which were resistant to red rot and mosaic virus. Haq *et al* (1974) obtained nine mutants of Co 633 and six of Co 527 which showed different degrees of resistance to *G tucumanensis*. The mutant Co 527-85 was resistant in both field and laboratory.

Single-bud setts of sugarcane were irradiated with 3 and 5 KR of gamma rays and tested for resistance to *G tucumanensis* in the M_2 generation. A considerable increase in resistance was observed (Haw *et al* 1974).

Single bud setts of varieties Co 419, Co 312, Co 527 and Co 602 were irradiated with

gamma rays at dose rate 3, 5, 6 and 8 KR and X-rays at dose rate 5, 7.5 and 10 KR. The variety Co 527 gave highest frequency of moderately resistant mutants in the VM_2 generation followed by Co 312 (Jagathesan *et al* 1974). Roach (1974) reported that Triton and Appolo cutting sub-clones when treated with 3 and 5 KR of gamma rays had significantly higher sugar content and lower fiber. Krishnamurthi and Tlaskal (1974) developed *in vitro* sugarcane lines that were resistant to Fiji disease virus through somaclonal variation and selected lines that had retained the high yield characterized by the parents.

Acute gamma irradiation effects at 1.0, 2.0, 3.0, 4.0, 5.0, 7.5, 10.0 kr, on sugarcane *(Saccharum* sp) clone Co 547 setts were studied through the grand growth period that included bud radiosensitivity estimation. Cane yield decreased significantly from 3·0 kR, chiefly due to low plant survival at maturity. Variants were selected for increased internode thickness, higher tiller number and rind colour changes. Variability can be induced in a high frequency in sugarcane following sett irradiation. However, in order to maintain a positive high yield, optimum sugar recovery and population size, the treatment limits should not exceed 3.0 kR (Siddiqui 1975).

Bhagyalaxmi (1975) studied the induction of mutations in sugarcane and many morphological changes were observed in plants at nine month stage in VM_1 generation. Jagathesan (1977) obtained mutations in a number of varieties in the Co series following mutagenic treatment of single bud setts, particularly for dwarfness, flowerlessness, high sugar content, glabrous leaves, increased growth rate and yield and resistance to *G tucumanensis*.

An erect leaved mutant of Co 740 and several mutants of Co 419 with increased stem diameter, individual cane weight and sucrose percentage were isolated by Rao *et al* (1977) when treated with 2 KR of gamma rays. A vigorous fast growing mutant with a shorter span of vegetative growth, sixty days earlier than the parent variety Co 527, was isolated from gamma irradiated vegetative buds. The mutant initially segregated but stabilized in the M_4 generation. Its growth rate was almost fifty per cent higher than of Co 527, beginning in the early stages of growth. It produced a significantly higher early shoot population which enabled it to yield a higher number of millable canes at maturity. However economic characters like sucrose content and juice purity remained unaffected (Jagathesan 1978).

Selim *et al* (1980) studied the effect of gamma -irradiation (3, 4, 5 or 6 kr) on singlebud cuttings of sugarcane cultivars NCo 310 and GT 54-9 was studied. Germination percentages decreased at all rates compared with untreated controls in both the cultivars. The number of internodes in NCo 310 increased significantly at all the doses in the plant crop but in GT 54-9 the increase occurred only with the 3 kR dose in the plant and first ratoon crops. Irradiation reduced leaf area at the low doses (3 and 4 kR) in the plant crop, but increased the number of tillers and millable canes.

Six commercial varieties of sugarcane under cultivation in India were subjected to

mutagenic treatment for inducing mutations (Jagathesan 1982). More than 50 mutants for various morphological characters, disease resistance and higher sugar content were obtained.

Single bud setts of sugarcane variety Co 1148 were irradiated with physical mutagens (gamma rays) and chemical mutagens (EMS and SA). The clones which survived were inoculated with red-rot isolate of Co1148. Mean, range and C.V % were estimated for brix number, sucrose percent, purity coefficient, commercial cane sugar, juice extraction % and fibre content in mutagenic population of sugarcane. Significant increase in mean values, range and C.V % was observed for most of quality characters as compared to control (Khairwal *et al* 1984).

Seven multiple-bud mutants, including one gall-forming mutant (US 94-12) were tested with two normal cultivars of sugarcane plant crops for 2 years in a randomized complete block design with four replications. Normal cultivars produced one shoot per node in the greenhouse. Most multiple-bud genotypes produced two shoots per node, except US 94-12 that produced 4.5 shoots per node. Development of a multiple-bud cultivar will be hindered by the low rate of transmission and therefore, the large population required for selection (Burner and Legendre 1997).

Three morphologically distinct mutants of M-1, M-2 and M-3 were isolated from 30,40 and 60 Gy of gamma rays treated plant population of sugarcane *cv* CoS 687 with the frequency of .01%, .04% and .01% respectively and found stable upto VM5 generation. Three mutants were found resistant to red rot (Singh *et al* 1999).

A study was carried by Khan *et al* (2000) out to develop mutants in sugarcane using *in vitro* mutagenesis technology. The calli were exposed to four radiation doses. The plants did not develop from calli exposed to dose of 6.0 Kr but developed well with lower doses. 0.5 Kr showed good effect on some agronomic characters i.e. plant height number of tillers per plant, cane thickness and number of green leaves per plant.

Kwon-Ndung and Ifenkwe (2000) conducted studies on sugarcane between 1994 and 1998 using gamma radiations. A dose sensitivity range of 4-8 Kgy were identified for optimum germination of cane buds. Growth and quality traits of first generation of vegetative mutants (M_1V_1) reduced when gamma dose increased. Relationship which was inconsistent in M_1V_2 showed stabilised effects of these traits in M_1V_3.

Majid *et al* (2001) irradiated three varieties of sugarcane, 'Isd-2/54', 'Nagarbari' and 'Latarijaba', with 20, 30 and 40 Gy gamma rays. Among the 2,954 MV3 hills, inoculated with red rot spore suspension, 37 resistant and 151 moderately resistant plants were isolated. In another experiment, four varieties of sugarcane, 'Isd-2/54', 'Isd-16', 'Nagarbari' and 'Latarijaba' were irradiated with 20, 40 and 60 Gy gamma rays. Approximately, 10,000 MV3 canes were planted in a low-lying field, and subjected to water-logging stress. MV5 and MV6 populations were inoculated with red rot spore suspension under waterlogged conditions. Five

MV5 variants were selected on the basis of greenness of the leaves, growth of the canes, number of nodes bearing adventitious roots, Brix index, cane yield and disease reactions, and grown as MV6 propagation. Three selected variants, SCM-12, SCM-14 and SCM- 15, were tolerant to waterlogged conditions in MV6. Two additional varieties, 'I-291/87' and 'I-525/85' were treated with 20, 30 and 40 Gy gamma rays to select for delayed/non-flowering types. Four variants were selected in MV3 for showing delayed flowering where as one mutant, SCM-28 flowered three months later than the parent 'I-291/87'

Sugarcane clones resistant to sugarcane mosaic virus (SCMV) were obtained using induction mutation by irradiating calli from the susceptible cultivar B6749 with 2 krads of gamma rays. The regenerated plantlets were tested for resistance to strain B of SCMV in the greenhouse and the resistant clones were transferred to the field. They have shown stable resistance to viral infection for eight clonal generations of (Zambrano *et al* 2003).

Seven week old well developed calli of sugarcane were either treated with different concentrations of sodium azide ranging from 1.0 to 5.0 mg/l or irradiated with 10 Gy, 20 Gy, 30 Gy, 40 Gy and 50 Gy doses of gamma-rays for induction of mutation. Partially purified toxin of *Colletotrichum falcatum* ranging from 0.05% to 0.5% was added in callus regenerating medium. Minimum plants were regenerated at 0.5% toxin with maximum callus death. These plants regenerated from callus which was insensitive to red rot toxin and were supposed to be disease resistant. These *in vitro* screened plants after rooting were hardened and acclimatized in the glass house and were shifted into the field. Field screening was carried out against two different isolates of *Colletotrichum falcatum* by using syringe method of inoculation (Ali *et al* 2007).

Vegetative cuttings of three sugarcane clones NI-98, NIA- 2004 and BLA were tested for induced somatic mutations using irradiation doses of 0, 10,20, 30, and 40 Gy. The dose 30 Gy and 40 Gy showed negative impact on agronomic traits but the dose 20 Gy had enhancing effect on plant height and cane yield (Khan *et al* 2007).

Sugarcane mutants against salt tolerance were derived using *in vitro* selection and regeneration under NaCl stress. Sugarcane cv Co 86032 was used for callus development from meristematic leaf whorl. Callus was treated with EMS, a potential mutagen at 0.5% for different time intervals of 1, 1.5, 2, 2.5 and 3 hours separately. Calli treated with EMS for 2 hours failed to induce any active regeneration. A maximum number of 15 healthy plants were successfully regenerated from the calli treated for 2.5 hours. Treatment of EMS for 3 hours induced regeneration at lower rate with three healthy plants only. Treatment for 2.5 hours was most ideal in deriving maximum mutants (Kenganal 2008).

Callus cultures were developed in three commercial sugarcane varieties viz, CoJ 64, CoJ 83 and CoJ 86 from spindle explants on MS + 2, 4-D (4 mg l^{-1}) + BAP (0.5 mg l^{-1}) medium. Callus and callus derived shoots were treated with gamma radiation at 20, 40, 60

and 80 Gy. Per cent shoot regeneration from gamma-irradiated calli in the three varieties ranged from 90 to 93.8 at 20 Gy, 83.3 to 87.5 at 40 Gy, 30 to 36.4 at 60 Gy and 0 at 80 Gy. Upon irradiating shoots, subsequent shoot proliferation in the three varieties ranged from 90.9 to 93.1% at 20 Gy, 82.6 to 84.0% at 40 Gy and 27 to 32.3% at 60 Gy, whereas 80 Gy dose was 100% lethal. Thus, 60 Gy dose of gamma-radiation was found to be optimum for carrying out mutagenesis of both callus and callus derived shoots. In the field, different irradiated clones of the same variety exhibited huge variability with respect to number of canes, cane girth, cane height and sucrose content (Kaur and Gosal 2009).

In vitro mutagenesis was employed in the selection of salt tolerant lines in popular sugarcane (*Saccharum officinarum* L) *cv* CoC 671,Co 86032 and Co 94012. Embryogenic cultures were gamma irradiated (10-50Gy) and challenged with different levels of NaCl (42.8 - 256.7 mM). Plant regeneration was observed in 10 and 20Gy irradiated calli up to 171.1 mM NaCl selection. A total of 147 plantlets were selected on different salt levels and the tolerant lines evaluated at field level. Plantlets regenerated form irradiated calli of sugarcane *cv* CoC-671, Co 86032 and Co 94012 were field planted and agronomically desirable variants were identified for economic traits like cane yield and sucrose (Suprasanna *et al* 2009).

In a study by Khan *et al* (2010) variability obtained from mutation breeding (gamma rays) in sugarcane was examined through molecular marker techniques (RAPD). A total of 85 loci were amplified, out of which 76.47% were polymorphic and 23.53% were monomorphic. Fragments size ranged from 220bp-2.1kb and fragments produced by various primers ranged from 3-13 with an average of 5 fragments per primer. The highest number of loci (13) was amplified with primer B-07, while the lowest number 3 with primer B-01. Results revealed that mutant P1 (20Gy) contained a specific segment of 2.03kb. Genetically most similar genotypes were P2 (10Gy) and P4 (20Gy) (95.55%) while most dissimilar genotypes were P4 (10Gy) and P3 (20Gy) (63.2%). On the basis of results achieved, the mutants could be divided into four clusters and three groups. Mutants P4 (40 Gy) and P4 (10 Gy) were genetically distinct from other mutants.

Three sugarcane clones viz., NIA-98, NIA-0819 and BL4 were used for induction of genetic variability through *in-vitro* mutagenesis. Apical meristametic region was used for callus induction on 4mg/l 2,4-D. Actively growing callus was treated with four different doses of gamma rays (10Gy, 20Gy, 30Gy and 40Gy). Maximum callus proliferation and plantlet regeneration and growth was observed in control and minimum at 40 Gy. Maximum chlorophyll mutation frequency was recorded in 30 and 40 Gy. Maximum number of roots were observed in control followed by 10 Gy. The maximum root length (10.3cm) was obtained in control at 2 mg/l IBA. The tiller number was maximum in the irradiated population at 10 Gy. The treatments 30Gy and 40Gy exhibited negative impact on tillering potential of the plants (Yasmin *et al* 2011).

In order to induce mutation in two commercial varieties of sugarcane in Iran viz. CP48-103 and CP57-614, ethyl-methane sulfonate (EMS) was utilized in different doses on calli. Callus cells of these varieties were exposed to EMS at different concentrations. Besides it implied the significance of regression coefficient at a=0.01. A regression equation for the relation between utilized dose of EMS and mortality of calli obtained was Y= -8.18 +5.28X (Sadat and Hoveize 2012).

A new sugarcane variety Guifu 98-296 was selected by mutation breeding by Song *et al* (2011). In different sugarcane growing test areas of Guangxi, Guifu 98-296 cane output was 118.95t/hm2, sugar output was 16.65t/hm2, and these were higher by 32.98% and 29.07%, respectively, than those of ROC 22the main variety in Guangxi. The differences between the two were highly significant. Guifu 98-296 grows quickly with middle stalks, strong resistance to insect and disease, good ratoon performance, and was found suitable for growing in the drought and slope upland of Guangxi Province.

The brown rust susceptible sugarcane genotype B4362 was subjected to *in vitro* tissue culture and physical and chemical mutation induction procedures. Five brown rust resistant mutants with hypersensitive response to *Puccinia melanocephala* were selected out of a total population of 11 167 regenerated plants. The brown rust resistant mutants showed variations in molecular, morphological, and agronomic traits. Sugar yield was improved in two mutants with increments in stalk length, stalk number, and stalk diameter (Maria *et al* 2012).

Mutation studies in other vegetatively propagated crops

Vasudevan *et al* (1967) opined that a radiation dose between 40-70 Gy was optimum for stem cuttings. Lapins *et al* (1969) reported that the irradiation of very young peach seedlings from embryo culture resulted in increased mutation frequencies with increasing dose. Singh and Iyer (1972) carried out an experiment to standardize dosimetry for young slips of cultivars Kew and Queen pineapple by irradiating with gamma rays from 1 to 200 kR. For both the cultivars 70 kR was found to be the LD_{50} dose.

Mutagenic treatments were applied at different stages previous to the development of adventitious buds of potato. Mutations were induced by ethyl methanesulphonate (EMS) and X-rays. The use of the adventitious-bud technique resulted in a considerable reduction of chimerism when X-rays were used. A dose of 2 krad of X-rays was optimal for the production high numbers of mutated plants (Miedema 1973).

Rhizomes of two turf bermudagrass cultivars were treated with gamma rays of dosages 9000 and 11,300 rads. Seventy one distinct mutations were screened in the treated material. The most frequent mutations observed were dwarfism and change in color hue of stolons (Powell *et al* 1973).

From shoot tip explants of taro (*Colocasia esculenta* (L.) Schott) plantlets were regenerated on a Murashige and Skoog medium treated with 0–20 Gy of gamma radiation.

These were acclimated in the greenhouse and cultivated in the field. No visible variation was observed among plants grown from seed corms and shoot tips treated with gamma radiation. A 12% rate of variation was observed among plants directly regenerated from the irradiated shoot tips. Among these plants, eight variants were selected and observed for various characters during the succeeding year. Increased number of lateral shoots, flowering behavior, increase or decrease in plant height, presence or absence of corms, unusually small and large leaf blades, variations in the amount of anthocyanin in petioles, late sprouting and late wilting were among the significant characteristics of the variants. Variants R28 and R51, which were dwarf types, and R56 and R152, whose corms could be separated easily from the main corm, were considered horticulturally valuable. Variants R12 and R15 did not produce corms, but extensive flowering occurred (Malamug *et al* 1974).

The seeds of Co 1 red gram (*Cajanus cajan (L) Millsp.)* were treated with gamma rays (0, 14, 16, 18, 20, 22, 24 and 26 kR) and EMS (0, 60, 70 and 80 mM for 4 hr at room temperature).Observations were recorded in the M_1 and M_2 generations. The chlorophyll mutations in M_2 were maximum at 24 kR of gamma rays and 70 mM of EMS, but the maximum frequency of viable mutations was noted around 16 kR and 60 mM. The percentage of viable mutations was found to be greater for gamma ray treatments compared to those for EMS. Some morphological mutants have been reported for the first time (Khan and Veeraswamy 1974).

Moh and Alan (1974) irradiated stem cuttings of cassava with upto 40 Gy gamma rays. The LD_{50} dose was approximately 30 Gy (1Gy= 100 rad). Mutant plants arising in the progeny of induced interchange heterozygotes of grain Sorghum variety I S 3566 were studied. The mutants were classified and considered under three different height classes of "2-Dwarf", "1-Dwarf" and "0-Dwarf". The "2-Dwarf" (tall) plants were observed to produce more grains than the "3-Dwarf" (short) controls. Other vegetative and panicle characters in the apparently true-breeding mutant were also affected including increased numbers of leaves, nodes and productive tillers. The mutant plant produced red grains on compact panicles of reduced length compared to chalky white grains on the semicompact panicles of the untreated controls. An increase in grain weight of about 21 per cent was shown in selfed progenies studied through successive generations (Rao and Reddi 1975).

Tuber eyes of Katahdin and B5141-6 potatoes were irradiated with doses below 4500 Rads X-rays or 1200 rads fast neutrons. Only slight reductions were observed in sprouting and growth. Survival was reduced about 50% by 1400 rads fast neutrons and 97% by 5500 rads X - rays (McCollum 1977).

Survival rate of apple seedlings was higher along with better mutation rate under water protection of basal part when one year old dormant shoots were treated with 5-10 kR gamma irradiation (Lacey and Campbell 1977). Das *et al* (1978) obtained 19 flower mutants

when dahlia tubers were irradiated with gamma rays up to 8 kR.

Stolon pieces of St. Augustine grass were irradiated with gamma rays in an attempt to cause mutations. Dosage for most genotypes was 4500 rads. This caused 50% growth retardation and a mean survival of about 40% of single -node cuttings. At this dose up to 7% recognisable mutants of accession FA-243 were obtained. This proportion was observed when irradiated cuttings were propagated clonally. A chimeric anthocyanin change too was noticed (Busey 1979).

Mutation frequency in diploid and autotetraploid of *Portulaca grandiflora,* Hook, following treatment of 2n and 4n cuttings with gamma-rays at 2, 4 and 6 krad was compared. Pointed leaf mutant in 4n at 4 krad revealed a case of somatic reduction (Raghuvanshi and Singh 1979). Gupta *et al* (1982) exposed rhizome pieces of *Costus speciosus* to 15,20, 25 and 30 Gy. Based on sprouting and survival, they concluded that the LD_{50} for *C speciosus* appears to be around 30 Gy and reported that six plants of *Costus* were selected for increased yield with higher or lower diosgenin content after irradiation of rhizomes with gamma rays. Besides, plants showing early flowering, dwarf and increased branching types were also selected for further study.

Rangaswamy (1986) while recording the achievements of mutation breeding research one distinctly dwarf mutant was obtained when scion sticks of Dashehari was subjected to 3.5 kR gamma rays whose fruit quality was similar to Dashehari, but fruit size was small (Sharma and Mujumdar 1988). For X rays and gamma rays dose range between 20-30 Gy is preferred by most workers for vegetative parts of potato. Doses of 80- 100 Gy are usually lethal (Broertjes and Van, H 1988). Sharma and Mujumdar (1988) subjected scion sticks of mango cultivars, Mallika, Dashehari, Amrapali and Neelum to a wide range of acute gamma irradiation. They reported that LD_{50} was 2.4, 2.9, 3.2 and 3.9 kR respectively, for these cultivars.

Mohanty and Panda (1988) conducted a mutagensis study in ginger by comparing physical (gamma rays) and chemical (EMS) mutagens. The LD_{50} value for gamma rays was between 0.5 to 1.5 kR whereas, it was between 0.9 to 1.5% for ethyl methane sulphonate (EMS). They opined that EMS was more effective in inducing chlorophyll and morphological mutations and both mutations proved effective in enlarging morphological variations in this vegetatively propagated crop. Critical dose for developing buds of sour cherry was 30 Gy (Zykov and Klimenko 1989).

For induction of mutation in banana cultivar Maca, an *in vivo* method was adopted in which rhizomes from adult plants were placed in sterile vermiculite medium in green house. Sensitivity of lateral buds from the rhizomes was assessed. LD_{50} was found to be 30-40 Gy (Neto *et al* 1989). Datta and Banerji (1990) reported that the size of stomata and stomatal index of bougainvillea were significantly reduced in gamma ray induced mutants. Roy *et al*

(1990) obtained one seedless mutant in M_1V_2 plants when bud wood of highly seeded lemon was exposed to 50 Gy gamma rays and buds from M_1V_2 plants budded on to sour orange.

Somatic mutations were induced by Banerji and Datta (1991) in chrysanthemum cultivar 'Anupam' after irradiation of rooted cuttings with 1.5, 2 and 2.5 kR gamma radiation. Coloured mutants were found in the M_1 population as chimeras as well as three full coloured flower mutants which were isolated and established as new cultivars.

Giridharan and Balakrishnan (1992) applied four different doses of gamma rays for irradiating ginger cultivars Rhio de Janeiro and Maran. The sprouting percentage of treated rhizomes was reduced by 50 per cent between doses of 1.5 and 2 kR.

Jayachandran and Kumar (1992) irradiated rhizomes of ginger *cv* Rhio de Janeiro with five doses viz 0.50, 0.75, 1.00, 1.25 and 1.50 kR utilizing Co60 gamma chamber. LD50 value based on surviving plants appeared to be between 1.0 and 1.25 kR. Jayachandran and Kumar (1992) observed chlorophyll chimera in ginger to a limited extent ranging from 2.5-6.5 per cent when irradiated with gamma rays. Though increased irradiation doses reduced different vegetative characters in ginger, flowering behaviour was not altered by the irradiation doses (Giridharan and Balakrishnan 1992).

Genetic and developmental effects of heavy ions in maize and rice were investigated. The frequency of occurrence of white-yellow stripes on leaves of plants developed from irradiated maize seeds increased linearly with dose, and high-LET heavy charged particles, e.g., neon, argon, and iron, were 2-12 times as effective as gamma rays in inducing this type of mutation. The effectiveness of high-LET heavy ion in inhibiting rice seedling growth, reducing plant fertility, inducing chromosomal aberrations and micronuclei in root tip cells and pollen mother cells of the first generation plants developed from exposed seeds, and inducing mutation in the second generation, were greater than that of low-LET gamma rays. All effects observed were dose-dependent; however, there appeared to be an optimal range of doses for inducing certain types of mutation, for example, for argon ions at 90-100 Gy, several valuable mutant lines with favorable characters, such as semidwarf, early maturity and high yield ability, were obtained. RFLP analysis of two semidwarf mutants induced by argon particles revealed that large DNA alterations might be involved in these mutants (Mei *et al* 1994).

Nwachukwu *et al* (1994) studied the radio sensitivity of black ginger (cv Vatsum Biri) and yellow ginger (cv Tafin Giwa) by irradiating at 2.5, 5.0, 7.5 and 10.0 Gy gamma rays. GR_{50} (50% growth reduction) values were 5 and 6 Gy for Tafin Giwa and Yatsum Biri, respectively, while, LD_{50} value was 8.75 Gy for both varieties.

Hemalatha (1998) observed several morphological changes in irradiated carnation plants. They included flower variegation, incurved petals, distorted petals, change in flower colour and small size flowers. These changes persisted through subsequent vegetative

generations and were considered to be cases of somatic mutation and survival percent of 49.96 at 3 kR and 58.20 at 2.5 kR indicating that LD_{50} value for carnation cuttings lies between those two doses. Hemalatha (1998) obtained three viable mutants of carnation cv. Sterilite Dop in VM2 generation viz, colour mutant with red colour, another colour mutant with reduced orange tinge at 1.5 kR and miniature flower mutant at 2.5 kR gamma rays.

Rao (1999) used 0.3, 0.6 and 0.9 kR gamma irradiation treatments in turmeric cv Mydukar. With increasing dosage of radiation germination, growth and yield per plant were reduced. While using gamma irradiation for turmeric reported that treatment with 0.6 kR resulted in an increase in curcumin content. He also observed that chlorophyll mutations were observed at the highest dose. Chezhiyan and Shanmugasundaram (2000) released a promising turmeric variety (BSR-2) which is the result of irradiation of Erode local with 10 kR X-ray. It has medium stature, short duration and 32.7 t/ha mean rhizome yield.

Axillary bud explants of carnation were subjected to mutagenic treatment with ethyl methane sulphonate under *in vitro* conditions. EMS at dose rate of 0.025 and 0.050 per cent in culture medium and 0.25 per cent used for explants, showed stimulatory effect on sprouting, number of shoots, number of flowers and flower initiation, while higher doses of 0.075 and 0.100 per cent under MS and 0.50, 0.75 and 1.00 per cent employed for EA caused damage to all vegetative and floral parts. Two colour mutants, red with white stripes and pink with white stripes were isolated from the mutagen treated population (Singh *et al* 2000).

Apical buds of lateral branches in rose, asexually multiplied by cuttings, were treated with chemical mutagens and the growth and differentiation of the mutagen treated buds were traced in developed flowers. It resulted in variation in size, shape, color and number of petals. Frequency was more in flowers that were developed from apical buds treated with N-methyl-N-nitro-N-nitrosoguanidine at 10 μgml^{-1}. The variant plants were cultured on MS medium. Regenerated plants produced varied flowers different from that originally used for tissue culture (Teruo *et al* 2001).

Sweetpotato cultivar Kokei No. 14 was planted in a ^{60}Co gamma field. Shoot apices from the plants irradiated with different doses were cultured on MS medium. Plantlets from somatic embryos developed into whole plants on the basal MS medium. The regenerated plants were transplanted to field and one mutant with alteration of root flesh color and yield was obtained. The root flesh color had changed from light-yellow in the wild-type plant to orange in the mutant. The yield of storage roots increased significantly in the original plant to mutant per plant. Carotenoids content of the storage roots in the mutant had significant difference compared to the wild-type at the 1% level (Wang *et al* 2007).

Mutants of bermudagrass were generated using gamma-ray irradiation with an aim toward developing dwarf and drought-resistant bermudagrass. Three mutant lines (7-9, 10-5 and 10-12) that exhibit slow growth and good turf quality were chosen for this study.

Bermudagrass mutants induced by gamma radiation exhibited dwarf characteristics and improved drought resistance, which was associated with maintenance of higher levels of antioxidant enzyme activities and non-enzymatic antioxidant contents. (Chen *et al* 2009).

Batches of 150 yam mini-tubers of a well adopted local cultivar "Obiaoturugo" were gamma irradiated at doses of 0, 10, 20, 30, 40, 50, 60, 70, 80 and 90 Gy. Each irradiated tuber including the control was divided into setts weighing 10-15g and planted in the field to MV1, separating setts from the head and the tail regions. At the MV1 generation, increasing doses of Gamma-ray irradiation progressively inhibited sprouting of setts isolated from treated mini-tubers. These effects were more severe on setts from the tail region than those from the head region. Also, plant height, number of leaves, number of nodes and mean tuber yields per stand decreased with increased gamma-ray doses. LD_{50} were observed at 40Gy. At the MV2 generation, the observed differences among the treatments means disappeared. However MV2 yam lines with modified vegetative characteristics were isolated (Nwachukwu *et al* 2009).

Using acute irradiation of Gamma-rays and in vitro cultured meristems, selection of resistant mutants to bacterial heart rot disease with improved fruit quality has been carried out in the most popular variety, Josapine meristem explants were irradiated with a series of Gamma-ray doses of 0, 20, 40, 60, 80, 100, 120, 140 and 160Gy and radiosensitivity was investigated based on shoot formation and survival rate. Radiosensitivity of meristem tissues producing shoots was inversely proportional to the irradiation dose. As the dose was increased mutations appeared more frequently, indicated by the formation of albino and striped leaves. The dose required for 50% lethality (LD50) and 100% (LD100) of meristem tissues for shoot formation were 40Gy and 83Gy respectively (Ibrahim 2009).

A wide range of viable morphological and physiological mutants were observed in M_2 and M_3 progenies of mungbean (*Vigna radiata* (L.) Wilczek) cultivars (Vaibhav and Kopargaon-1) raised from seeds treated with different concentrations of sodium azide, ethyl methane sulfonate and different doses of gamma radiation in a study by Auti and Apparao (2009).The most striking type of mutants obtained in the M3 progeny included plant habit, leaf structure, flower type, pod type, seed type, early-maturing and high-yielding and Lhb mutants. These mutants could be better fitted in new cropping patterns, with improved agronomic management and good yielding ability, or can be used in the genetic improvement of mungbean crop.

Mutagenic effectiveness and efficiency of gamma rays, EMS and their combined treatments were studied in the genotype of cowpea variety CO 7. The mutagenic treatments seeds were tested for lethal dose 50 percent for all mutagens. The frequency of mutation was more in combined treatments than gamma rays and EMS. The mutagenic effectiveness and efficiency was calculated based on biological damage. In M_1 and M_2 generations, based on seed lethality (L) and seedling injury (I) were carefully screened for various chlorophyll and

viable mutations. Mutagenic effectiveness and efficiency increased with the decreased in dose or concentration. In the present study EMS was provide to be more effective and efficient in causing mutations as compared to gamma rays and the combined treatments (Girija and Dhanavel 2009).

Seed tubers from selected genotypes were grown in the primary nursery and 45-day-old single node cuttings were taken for mutagenic treatment using physical and chemical mutagen selected doses for gamma irradiation were 10, 20, 30, 40, & 50Gy and of EMS were 0.2, 0.4, 0.6, 0.8 and 1.0 %. The response of Coleus to mutagen varied with concentration as well as with genotype. Mutation has changed the plant height, size of tubers and tubers per plant. In addition, selected mutants showed photoinsensitivity to tuberization and acceptable qualitative changes (Abraham and Radhakrishnan 2009).

Drought is one of the major environmental stresses which greatly affects the plant growth and productivity. In the present study, various doses (0–75 Gy) of gamma rays were applied to investigate the effect of radiation on shoot tip explants. It was observed that the regeneration rates and plant fresh weights decreased significantly with an increase in radiation dose. The optimal irradiation doses for mutation induction were determined as 15 and 20 Gy. Afterwards, the induction of somatic mutation in sugar beet (*Beta vulgaris* L.) was investigated by irradiation of shoot tips with 15 and 20 Gy gamma rays (Sen and Alikamanoglu 2012).

The effect of gamma rays on germination, morphological yield and yield components characters in cowpea was studied by treating the seeds with different doses of gamma rays (0 control, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50KR), the morphological, yield and yield components characters days to first flower, plant height, number of leaves per plant, number of branches per plant, number of pods per plants, number of seeds per plant, 100 seeds weight and yield per plant decreased with increased with increasing dose of gamma rays were recorded (Gnanamurthy *et al* 2012).

Gamma Chamber

PLATE 1

Refractometer

PLATE 2

CHAPTER - III

MATERIALS AND METHODS

Experimental material

The experimental plant material used in the present study consisted of an important sugarcane variety Co J 64 having desirable attributes like early maturity, high sugar and cane yield but susceptible to red rot caused by *Colletotrichum falcatum*. The variety was subjected to mutagenic treatment using gamma rays generating variability. About 1200 single buds were irradiated as **shown** in Table 1 below:

Table 1: Different irradiation treatment combination used in study

Variety	No. of sets	Treatment dose (Gy)	Duration
Co J 64	1200	10	1 min 17 sec
	1200	20	2 min 34 sec
	1200	25	3 min 13 sec
	1200	30	3 min 52 sec

Methods

Priming

Single buds of sugarcane variety Co J 64 were subjected to water priming (primed and non-primed) prior to their irradiation in gamma chamber.

a) Priming of buds **was** done by dipping the buds in water for 12 hours prior to irradiation. After that buds were taken to gamma chamber for irradiation and were irradiated at varying dose of gamma rays for different durations.

b) Non-primed buds i.e., fresh cut material was directly taken to gamma chamber for irradiation and treated at varying doses of gamma rays.

Mutation Treatment

Somatic tissues (buds) of sugarcane were irradiated for generating variability. Twelve hundred single bud setts of Co J 64 were irradiation with gamma rays at aforementioned doses in gamma chamber (Blood Irradiator 2000) shown in (plate 1) located at Department of Horticulture, Punjab Agricultural University, Ludhiana. Fifty buds were treated at a time in gamma chamber for different time intervals *viz.* 1 min 17 sec, 2 min 34 sec, 3 min 13 sec and 3 min 52 sec for 10, 20, 25 and 30 Grays (1 Gy = 1 Joule kg^{-1}), respectively. The material generated was used to grow first (M_1V_1) generation by following two priming methods.

Planting of Experimental Material

Irradiated material (M_1V_1) obtained from different treatments was planted in the Sugarcane experimental area of Punjab Agricultural University, U.S.F, Ladhowal during spring 2012 for its evaluation under field condition. All the material was in planted in

Complete Randomized Design having plot size of 10.5m ×2.25m with intra-row spacing of 30 cm comprising 34 buds per row. Irradiated buds were treated with Methoxyethylmercuricchloride fungicide by dipping before sowing. Recommended package of practices were followed top to raise the healthy crop stand.

Canes from all the treatments were harvested separately and used to raise the second generation (M_1V_2) at PAU, Regional Research Station Kapurthala during spring 2013. The plot size comprised of 10 m x 3.65 m in complete randomized design. Single buds were treated with Methoxyethylmercuricchloride fungicide by dipping before sowing. Recommended package of practices were followed to raise the healthy crop stand.

Observations Recorded:

The data were recorded independently for different traits during both 2012 and 2013 seasons as detailed below:

Germination (%)

The number of bud germinated per treatment after 45 days of planting were counted and percent germination was calculated as:

$$\text{Germination (\%)} = \frac{\text{No. of buds germinated}}{\text{Total number of buds planted}} \times 100$$

Survival (%)

The number of plantlets survived after 120 days under field conditions was counted and percent survival was worked out as:

$$\text{Survival (\%)} = \frac{\text{No. of plantlets survived after 120 days}}{\text{No. of buds germinated}} \times 100$$

Number of Tillers per Clump

The number of tillers from 10 randomly selected clumps from each treatment was counted in the first week of August when first few internodes were developed. Average number of tillers per clump was calculated.

Number of Millable Canes per Clump

The number of effective millable canes from 10 randomly selected clumps of each treatment was counted. Average number of millable canes per clump was calculated.

Cane Height (cm)

The height of 10 randomly selected millable canes from different clumps for each treatment was measured above the ground level upto the top most visible transverse mark, when the growth was complete. Then the average height was calculated for each treatment.

Cane Thickness (cm)

The cane thickness was measured with the help of vernier calliper at the middle place of cane. Ten canes were selected at random and thickness of canes was measured for each treatment. The average thickness was calculated for each treatment.

Single Cane Weight (g)

Weight of randomly selected 10 canes per treatment was weighed separately using digital spring balance and average was worked out.

Leaf Length (cm)

The fourth leaf of the cane from the top most visible transverse mark was measured in centimetres from the base of the leaf blade to the apex of leaf. Leaves from ten canes were selected randomly and length was measured for each treatment. Average leaf length was calculated for each treatment.

Leaf Width (cm)

The width of the fourth leaf from the top most visible transverse mark of the cane was recorded from the centre of the leaf where the cane leaf has its maximum width. Width of ten randomly selected leaves from each treatment was measured and average was calculated.

HR Brix (%)

Brix was determined by using a hand refractometer (Erma, Japan) with scale ranging from 0 to 32˚ B for sugarcane juice (AOAC) (Plate 2), working on the principle that light entering a prism has a unique characteristic. That characteristic is represented by a value on a scale in units known as ºBrix. When light enters a dry prism, the field of view in an analog refractometer remains blue.

Statistical analysis

The data recorded were analyzed according to completely randomized design analysis (Snedecor and Cochran 1967) using statistical software CPCS-1 package developed by Cheema and Singh (1990). The significance of variation among the treatments was compared by applying 'F' test and critical difference (CD) at 5 percent level of significance. The analysis of variance for each trait was based as per linear model suggested by Fisher (1954).

$$Yij = \mu + gi + eij$$

Where,

Yij= phenotypic observation of ith genotype in the jth replication.

μ= general population mean

gi= effect of ith genotype

eij= error associated with ith genotype in the jth replication.

On the basis of this linear model the analysis of variance was done as follows:

Analysis of variance for CRD

Source of variation	Degree of freedom	Mean square	Expected Mean square
Treatment	(t-1)	Mt	$\sigma^2e + k\,\sigma^2t$
Error	(N-t)	Me	σ^2e

Where:

t = number of treatments;

$\sigma^2 t$ = variance due treatments;

$\sigma^2 e$ = error variance

k = no. of repeats

The standard error of mean SE(m) and critical difference (CD) for comparison of means of any two treatments were computed as follow:

SE (m) = $\pm\sqrt{Me/r}$

SE (d) = $\pm\sqrt{2Me/r}$

Critical difference (CD) = SE (d) x t value at 5% error degree of freedom

Analysis of variance for the factorial analysis:

Linear model

Yijk = μ + Ai + Bj + (AB) ij + eijk

Where,

Yijk = phenotypic observation of ith level of factor A, jth level of factor B for the kth replication.

μ = general population mean

Ai = effect of ith level of factor A

Bj = effect of jth level of factor B, and

(AB)ij = interaction effect of the ith level of factor A and jth level of factor B

Eijk = random error on the basis of this linear model the analysis of variance was done as follows

Analysis of variance for RBD (Factorial)

Source	Degree of freedom	Mean square	Expected MS
Treatment	Pq-1	Mt	σ^2 e+ b σ^2 AB + bqσ^2A+bpσ^2B
Factor A	p-1	Ma	σ^2 e+ b σ^2 AB + bqσ^2A
Factor B	q-1	Mb	σ^2 e+ b σ^2 AB ++bpσ^2B
AxB	(p-1) (q-1)	MaxMb	σ^2 e+ b σ^2 AB
Error	(b-1) (pq-1)	Me	σ^2 e

Where,

b = number of replications

p = level of 1st factor (A)

q = level of 2nd factor (B)

The standard error of mean SE (m) ± and critical difference CD for comparing the means of any two treatments were computed as follows:

SE (m) A = ± √ Me/bq

SE (m) B = ± √ Me/bp

SE (m) Ax B = ± √ Me/b

Inoculation under field conditions using plug method

PLATE 3

SE (d) A = ± √ 2Me/bq

SE (d) B = ± √ 2Me/bp

SE (d) A x B = ± √ 2Me/b

Critical Difference (CD) = SE(d) x t (5%) value at error degree of freedom

Evaluations of irradiated sub clones against red rot disease

The experimental material generated through different mutation treatments in present study was planted at PAU, RRS Kapurthala during spring 2013 (M_1V_2 generation) was evaluated for red rot resistance.

Methods:

Multiplication of inoculum

Red rot pathotypes Cf 08 (from CoJ 84) and Cf 09 (from CoS 767) were multiplied on oat meal agar medium in Petri dishes at 25±1ºC. For inoculations, freshly sporulating 7-10 days old cultures were used. The spores were washed with sterile distilled water and homogenized by shaking ; spore suspensions with concentration of $2X10^4$ conidia ml^{-1} **were** maintained.

Planting time and inoculation

Experimental plant material was planted on 25-03-2013 in the field area using single bud setts. Ten canes per treatment were inoculated by a suspension of two pathotypes viz. Cf 08 and Cf 09 prevalent under Punjab conditions by artificial inoculation under field conditions using plug method (Srinivasan and Bhatt, 1961) (Plate 3).

The inoculations were done in the third internode from the base of the standing canes injecting 1.0 ml of spore suspension ($2X10^4$ conidia ml^{-1}) with the help of hypodermic syringe. The core was then replaced and the opening finally sealed with modeling clay.

Disease scoring

Disease data were recorded after 60 days of inoculation. The condition of the top was

Scoring of the disease:

Sr. No.	***Criteria of grading***	**Score**			
1	Condition of the top (CT)	**Green (0)**		**Yellow/ dry (1)**	
2	Lesion width (LW) (Symptom on internode just above the inoculated cane)	**Nil (0)**	**Upto 25% area (1)**	**Upto 50% area (2)**	**>50% area (3)**
3	White spots (WS)	Nil (0)	Restricted (1)		Enlarged(2)
4	Nodal transgression (NT) (Average no. of 3 setts are taken)	Number of nodes transgressed			
		(0)	(1)	(2)	(≥3)

Disease grade = CT+LW+WS+NT

A* Score		**B**Reaction**
0-4.0	:	R (Resistant)
4.1-6.0	:	X (Intermediate)
6.1-9	:	S (Susceptible)

recorded and the canes splitted longitudinally. Observations were recorded on the number of internodes transgressed by the pathogen. The canes were rated 0-9 as per scale of Srinivasan and Bhatt (1961).

CHAPTER - IV

RESULTS AND DISCUSSION

The experimental plant material of present study entitled ‘ Irradiation induced mutagenesis in sugarcane’ M_1V_1 generation obtained from different treatments was planted in the Sugarcane experimental area of Punjab Agricultural University, U.S.F, Ladhowal during spring 2012 and second generation (M_1V_2) at PAU, Regional Research Station Kapurthala during spring 2013 for its evaluation under field condition. Treatment and observations were made as per the details given under material and methods. The results obtained under different aspects are presented below in the light of relevant literature.

Analysis of variance

Analysis of variance revealed non-significant differences among priming treatment for all the traits studied. However, mutation treatments revealed significant differences for germination, number of tiller/ clump, number of millable cane/ clump, cane height, HR brix, Leaf length and single cane weight while non significant for cane girth and leaf width (Table 2) of sugarcane in M_1V_1 sub clones of Co J 64 variety grown during spring 2012. Similarly, in M_1V_2 sub clones grown during spring 2013 at RRS Kapurthala significant differences exist for many traits except no. of tiller / clump, no. of millable cane/ clump and cane girth (Table 3) indicating the potential of mutagenic treatments for creating genetic variability for different traits in sugarcane crop.

Table 2. Analysis of variance for different traits in sugarcane (M_1V_1) sub clones of Co J 64 at USF, Ladhowal during spring season 2012

Traits		Mean Square due to			
	Source of Variation	Treatment	Priming	Treatment x Priming	Error
	Degree of freedom	4	1	4	20
Germination (%)		330.19*	177.63	33.96*	4.33
Survival (%)		1600.63*	625.63	5.30	12.90
No. of Tiller/ clump		7.10*	0.53	0.33	0.23
No. of Millable cane/ clump		4.80*	0.53	0.33	0.40
Height (cm)		431.90*	52.50	1.30	12.70
Girth (cm)		0.79	0.31	0.20	0.15
HR brix (%)		2.20*	0.20	0.10	0.94
Leaf length (cm)		69.90*	0.97	0.41	9.60
Leaf width (cm)		0.62	0.75	0.28	0.67
Single Cane wt. (g)		38570.25*	79.56	356.65	395.75

* Significant difference at 0.05 level of significance

These results are in agreement with the findings of Kwon-Ndung and Ifenkwe (2000) who reported non-significant differences in the tiller population in subclones evaluated in

M_1V_2 generation and significant differences for single stalk weight and HR brix. Earlier studies (Chaudhary 1971 and Premsekar and Appadurai 1981) also documented similar observation as reported in present study. The mean performance of different traits evaluated in M_1V_1 and M_1V_2 subclones of Co J 64 during 2012 and 2013 respectively are discussed in the following sections.

Table 3. Analysis of variance for different traits in sugarcane (M_1V_2) sub clones of Co J 64 at RRS, Kapurthala grown during spring season 2013

Traits		Mean Square due to			
	Source of Variation	**Treatment**	**Priming**	**Treatment x Priming**	**Error**
	Degree of freedom	**4**	**1**	**4**	**20**
Germination (%)		338.19*	180.63	38.96*	6.33
Survival (%)		1596.63*	620.63	4.95	11.90
No. of Tiller / clump		0.66	0.33	0.33	0.46
No. of Millable cane/ clump		2.30	0.30	0.13	0.63
Height (cm)		647.70*	204.50	37.90	120.10
Cane girth (cm)		0.12	0.94	0.29	0.17
HR brix (%)		2.30*	0.36	0.15	0.36
Leaf length (cm)		181.20*	56.70	17.70	10.40
Leaf width (cm)		0.62*	0.75	0.28	0.67
Single Cane wt. (g)		42700.33*	84.53	374.20	421.66

* Significant difference at 0.05 level of significance

Evaluation of M_1V_1 irradiated sub-clones

Results of the field tested M_1V_1 irradiated clones of Co J 64 during 2012 showed differential behavior of treatments for different traits (Table 4). The mean performances of various traits for different treatment combinations recorded in present study are detailed below:

Germination (%)

The per cent germination of irradiated buds after 45 days of planting were recorded for different treatments and presented in Table 4. Analysis of variance revealed significant difference for this trait among mutation treatments and treatment x priming interactions. The germination percentage of irradiated buds ranged from 27.50 to 43.16 per cent in M_1V_1 generation grown during spring 2012. The highest germination was (43.16%) for control closely followed by mutation dose of 10 Gy. Germination percentage decreased with increase in gamma rays dose the lowest being for 30 Gy. On the other hand there was non significant effect of water priming on this trait. Among different treatment combination non-priming with 10 Gy irradiation recorded the high germination percentage (40%) which was comparable to control (45%). Differential response of mutagenic treatment on single bud germination in sugarcane has also been reported by earlier workers (Kwon-Ndung and Ifenkwe 2000). The present results indicate that higher doses of gamma rays delay and

Table 4: Performance of important characteristics of M_1V_1 irradiated sub-clones of Co J 64 grown during spring 2012

Traits	Priming	Mutagen Dose (Gy)					Mean	CD (5%)
		0	10	20	25	30		
Germination (%)	P	41.33	39.66	32.00	30.00	30.00	34.59	T=2.50, P=NS, TxP=3.54
	NP	45.00	40.00	31.00	26.00	25.00	33.4	
Mean		**43.16**	**39.83**	**31.50**	**28.00**	**27.50**		
Survival (%)	P	90.00	74.66	63.33	53.00	52.00	66.59	T=4.76, P=2.78, TxP=8.76
	NP	82.00	63.00	55.33	50.66	43.33	58.86	
Mean		**86.00**	**68.83**	**59.33**	**51.83**	**47.66**		
Tillaer/clump	P	5.30	4.60	3.60	3.00	2.60	3.82	T=0.58, P=1.58, TxP=3.54
	NP	5.00	4.30	3.30	3.00	2.30	3.58	
Mean		**5.15**	**4.45**	**3.45**	**3.00**	**2.45**		
Millable canes/clump	P	4.30	3.60	3.00	2.60	2.00	3.10	T=0.76, P=1.58, TxP=3.54
	NP	4.00	3.30	3.00	2.30	1.60	2.84	
Mean		**4.15**	**3.45**	**3.00**	**2.45**	**1.80**		
Cane height (cm)	P	180.4	160.9	151.70	140.50	185.30	163.76	T=5.70, P=1.58, TxP=3.54
	NP	182.40	157.70	147.60	141.70	186.60	163.2	
Mean		**181.40**	**159.30**	**149.65**	**141.10**	**185.95**		
Cane girth (cm)	P	2.30	2.00	1.90	1.90	1.90	2.00	T=0.15, P=1.58, TxP=3.54
	NP	2.10	2.0	1.90	1.90	1.90	1.96	
Mean		**2.20**	**2.00**	**1.90**	**1.90**	**1.90**		
Leaf length (cm)	P	113.70	114.40	111.90	110.50	106.30	111.36	T=3.70, P=1.58, TxP=3.54
	NP	113.60	114.80	113.30	109.60	105.70	111.40	
Mean		**113.65**	**114.60**	**112.60**	**110.05**	**106.00**		
Leaf width (cm)	P	4.00	4.20	3.93	3.66	3.30	3.81	T=0.31, P=1.58, TxP=3.54
	NP	3.93	4.10	3.60	3.66	3.30	3.71	
Mean		**3.96**	**4.15**	**3.76**	**3.66**	**3.30**		
HR brix (%)	P	23.00	22.60	22.20	22.50	23.80	22.82	T=0.37, P=1.58, TxP=3.54
	NP	23.40	23.80	22.20	22.30	23.60	23.06	
Mean		**23.20**	**23.20**	**22.20**	**22.40**	**23.70**		
Single cane weight (g)	P	961.66	881.66	826.66	795.00	726.66	838.32	T=24.73, P=1.58, TxP=3.54
	NP	931.66	871.60	826.66	791.66	720.00	828.31	
Mean		**946.66**	**876.63**	**826.66**	**793.33**	**723.33**		

NP- non priming, T- treatment, P-priming, T x P- treatment x priming

suppress germination of sugarcane buds. These results are in complete agreement with the findings of Nwachukwu *et al* (1990).

Survival (%)

The number of plantlets survived after 120 days under field conditions was recorded in this study for different irradiation and priming treatments. Mean percent survival MiV1 generation is depicted in Table 4. It showed that there were significant differences among treatments for per cent survival rate under both primed and non-primed conditions. The highest per cent survival was recorded for control 0 Gy (86 %) and minimum for 30 Gy (47.66%). Further, mean per cent survival was higher in primed buds (66.59%) as compare to non-primed (58.86). Of the different treatment combinations (TxP) interactions survival percentage ranged from 43.33% to 90.00%. Effect of doses of gamma rays and ethyl methane

sulphonate on germination and survival of induced mutations in pigeon pea has been documented by Premsekar and Appadurai (1981) who reported the reduction in survival with the increase in gamma dose.

Number of tillers per clump

Analysis of variance revealed significant differences among mutation treatments when the buds were primed in water for 12 hrs (Table 2 and 4). The mean number of tillers per clump ranged from 2.60 to 5.30 with an average of 3.80 when the buds were primed in water for 12 hrs where as it ranged from 2.30 to 5.00 when non primed fresh cut buds were sown after irradiation treatment. Among irradiation treatments highest number of tillers per clump was recorded in control (5.15) followed by 10 Gy (4.45) and minimum in 30 Gy (2.45). These results are in agreement with the results of Yasmin *et al* (2011) who induced genetic variation in sugarcane through mutagenesis with doses of gamma rays 10, 20 30 and 40 Gy. They reported that different irradiation doses showed significant impact on average number of tillers, as the dose increased the tillering potential decreased. In the present study no significant differences were found for primed and unprimed treatments.

Number of millable canes per clump

Total number of tillers per clump does not reflect truly to number of millable canes but it is a general observation that a plant having higher number of tillers usually has a high number of millable canes. In this study, analysis of variance exhibited significant differences for this trait among different doses of gamma rays applied to the sugarcane buds. The mean number of millable canes per clump ranged from 2.00 to 4.30 with an average of 3.10 in the primed buds but it ranged from 1.60 to 4.00 in unprimed treatment with an average of 2.80. Mean number of millable canes was observed to be highest in control (4.15) followed by 10 Gy (3.30) and minimum in 30 Gy (1.60) having similar trend same as observed for number of tillers per clump (i.e. tended to drop with an increase dosage). Various factors such as inhibition of auxin synthesis (Gurdon 1954), production of diffusible growth retarding substances (Mackey, 1951) and inhibition of DNA synthesis (Gaul 1970) have all been reported to be able to effect reduced growth in irradiated plants or seeds.

Cane height (cm)

Significant differences among the different mutation treatments were observed from the analysis of variance (Table 2). Average cane height among different irradiation treatments ranged from 140.50 to 185.30 cm in water primed buds (Table 4). The mean height was recorded to be 163.80. However, in unprimed treatment height ranged from 141.6 to 186.6 cm for different mutation treatments. Highest cane height of 186.6 cm was observed in 30 Gy. Khan et *al* (2009) have also reported higher cane height in gamma ray irradiated clones of sugarcane than control. They observed significant differences among treatment for this trait and recorded maximum plant height for gamma ray treatment of 20 Gy. They also found

maximum cane height in clones of sugarcane variety NIA-2004 when mutagenic treatment was applied at the rate of 30 Gy. In present study, the dose of 30 Gy showed stimulating and enhancing effect on plat height which is in complete agreement with earlier findings of Khan *et al* (2007) who reported the genetic variability in sugarcane induced through mutation breeding.

Cane girth (cm)

There are significant differences for cane girth among the different mutation treatments in sugarcane buds evaluated during spring 2012 (Table 2). Average cane girth in different mutation treatments ranged from 1.90 to 2.30 cm when the buds were primed for 12 hrs in water and girth ranged from 1.90 to 2.30 cm in non primed buds (Table 4). There were no significant differences found for priming and non priming of sugarcane buds in water. Highest cane thickness of 2.30 cm was recorded in untreated population followed by 10 Gy (2.00 cm). Earlier these types of results were obtained by Khan *et al* (2007). In this study, higher mutation did not reflect significant difference in cane thickness in mutated population for doses of 20, 25 and 30 Gy.

HR Brix (%)

For refractometer brix the results were significantly different for different doses of gamma irradiation in sugarcane buds treated to induce genetic variability. Refractometer brix in different mutation treatments used in present study ranged from 22.20 to 23.80 percent in primed buds with an average of 22.80 and which is at par with the values of brix in non primed buds (Table 4). Maximum refractometer brix (23.80%) was recorded for 30 Gy treated population followed by control (23.40). Minimum refractometer brix (22.20) was recorded in population treated with gamma ray at the rate of 20 Gy. Similar to present results Khan et *al* (2009 and 2007) recorded low sucrose (%) and CCS (%) in 20 Gy and high in 40 Gy and 50 Gy, respectively.

Leaf length (cm)

Significant differences among mutation treatments were observed for leaf length in the study for M_1V_1 sub clones during spring 2012 (Table 2). Mean value of leaf length for different treatments varied from 106.30 to 114.40 cm with an average of 111.40 cm in primed and 105.70 to 114.80 cm with an average of 111.00 cm in non primed treatment (Table 4). Maximum leaf length (114.80 cm) was recorded in 10 Gy treated population followed by control**.** These results are in accordance with the findings of Hegde (2006) who studied effect of gamma irradiation dose on the vegetative characters in Turmeric (*Curcuma longa* L.) and reported that lower irradiation dosages have enhancing effect on leaf length. In this study, minimum dose i.e. 10Gy showed the maximum leaf length followed by the control.

Leaf width (cm)

Analysis of variance exhibited non significant differences among the different

mutation treatments for leaf width in this study for M_1V_1 sub clones (Table 2). Average value of leaf width ranged from 3.30 to 4.20 cm among the different mutagenic treatments in the irradiated material when priming was done prior to irradiation while it ranged from 4.10 to 3.30 cm in the unprimed irradiated buds (Table 4). Maximum leaf width was observed in 10 Gy dose (4.2 cm) which is at par with control (4.00 cm).

Cane weight (g)

Cane weight is the ultimate trait in sugar cane breeding programme. Due to low genetic variability in this crop exerted efforts are being made to enhance variability using different approaches. Perusal of data appended in (Table 2) revealed that significant difference exist for this important trait among different mutagenic treatments. Single cane weight in M_1V_1 irradiated sub-clones of variety Co J64 ranged from 726.66 to 961.66 g in primed buds while the corresponding values for unprimed buds were 720.00 to 931.66 g (Table 4). Among various mutagenic treatments maximum single cane weight was recorded for control closely followed by 10 Gy and 20 Gy. The higher doses have retarding effect on this trait. Significant high primary stalk weight for lower gamma irradiation dose (20 Gy) as compare to 40 Gy has also been reported by Khan *et al* (2007) in sugarcane which is in complete agreement with present results.

Variability for different traits in irradiated M_1V_2 sub-clones of Co J 64

The harvest of M_1V_1 irradiated subclones grown during spring 2012 was further evaluated during spring 2013 at PAU, Regional Station, Kapurthala for stabilization of different traits at this level of screening. The results obtained on various parameters are presented in Table 5 and described below:

Germination (%)

The per cent germination of irradiated buds for M_1V_2 generation after 45 days of planting were recorded for different treatments and presented in Table 5. Analysis of variance revealed significant difference for this trait among mutation treatments and treatment x priming interactions. The germination percentage of irradiated buds ranged from 27.50 to 46.66 per cent in M_1V_2 generation grown during spring 2013. The highest germination was (46.66%) for control closely followed by mutation dose of 10 Gy (40.83%). Germination percentage decreased with increase in gamma rays dose the lowest being for 30 Gy. On the other hand there was non-significant effect of water priming on this trait. Among different treatment combination non-priming with 10 Gy irradiation recorded the high germination percentage (39%) which was comparable to control (45%). Differential response of mutagenic treatment on single bud germination in sugarcane has also been reported by earlier workers (Kwon-Ndung and Ifenkwe 2000). The present results indicate that higher doses of gamma rays delay and suppress germination of sugarcane buds. These results are in complete agreement with the findings of Nwachukwu *et al* (1990).

Table 5 Performance of important characteristics of M_1V_2 irradiated sub-clones of Co J 64 grown during spring 2013

Traits	Priming	Mutagen Dose (Gy)					Mean	CD (5%)
		0	10	20	25	30		
Germination (%)	P	48.33	42.66	38.00	35.00	31.00	38.79	T=2.50, P=NS, TxP=3.23
	NP	45.00	39.00	34.00	29.00	25.00	34.4	
Mean		**46.665**	**40.83**	**36.00**	**32.00**	**27.50**		
Survival (%)	P	90.00	84.66	73.33	63.00	52.00	72.59	T=4.32, P=Ns, TxP=5.35
	NP	88.00	73.00	65.33	60.66	48.33	67.06	
Mean		**89.00**	**78.83**	**69.33**	**61.83**	**50.16**		
Tiller/clump	P	4.30	4.30	4.30	4.00	3.60	4.10	T=NS, P=NS, TxP=NS
	NP	4.60	4.30	4.30	4.00	3.60	4.16	
Mean		**4.45**	**4.30**	**4.30**	**4.00**	**3.60**		
Millable canes/clump	P	4.00	3.60	3.60	3.60	2.30	3.42	T=0.95 P=NS, TxP=3.54
	NP	4.00	3.60	3.30	3.00	2.30	3.24	
Mean		**4.00**	**3.60**	**3.45**	**3.30**	**2.30**		
Cane height (cm)	P	180.20	168.30	158.30	166.60	182.70	171.22	T=13.2, P=NS, TxP=4.75
	NP	183.30	178.50	157.40	177.20	185.80	176.44	
Mean		**181.75**	**173.40**	**157.85**	**171.90**	**184.25**		
Cane girth (cm)	P	2.10	2.30	2.30	2.30	2.30	2.26	T=NS, P=NS TxP=Ns
	NP	2.30	2.20	2.30	2.10	2.40	2.26	
Mean		**2.20**	**2.25**	**2.30**	**2.20**	**2.35**		
Leaf length (cm)	P	119.10	117.50	114.30	111.80	108.80	114.30	T=3.80, P=2.40, TxP=3.65
	NP	118.10	115.70	112.70	111.30	100.00	111.56	
Mean		**118.60**	**116.60**	**113.50**	**111.55**	**104.40**		
Leaf width (cm)	P	4.00	4.20	3.90	3.60	3.30	3.80	T=0.31, P=NS TxP=0.16
	NP	3.90	4.10	3.60	3.60	3.30	3.70	
Mean		**3.95**	**4.15**	**3.75**	**3.60**	**3.30**		
HR brix (%)	P	23.10	22.10	21.00	21.80	21.60	21.92	T=0.72 P=NS, TxP=Ns
	NP	22.30	22.00	20.90	21.90	21.50	21.72	
Mean		**22.70**	**22.05**	**20.95**	**21.85**	**21.55**		
Single cane weight (g)	P	975.66	981.66	876.66	795.00	706.66	867.12	T=28.75, P=1.70, TxP=4.54
	NP	961.66	971.60	826.66	780.66	710.00	850.11	
Mean		**968.66**	**976.63**	**851.66**	**787.83**	**708.33**		

NP- non priming, T- treatment, P-priming, T x P- treatment x priming

Survival (%)

The number of plantlets survived after 120 days under field conditions was recorded in this study for different irradiation and priming treatments. Mean percent survival M_1V_2 generation is depicted in Table 5. It showed that there were significant differences among treatments for per cent survival rate under both primed and non-primed conditions. The highest per cent survival was recorded for control 0 Gy (90%) and minimum for 30 Gy (48.33%). Further, mean per cent survival was higher in primed buds (72.59%) as compare to non-primed (67.06). of the different treatment combinations (TxP) interactions survival percentage ranged from 48.33% to 90.00%. Effect of doses of gamma rays and ethyl methane sulphonate on germination and survival of induced mutations in pigeon pea has been documented by Premsekar and Appadurai (1981) who reported the reduction in survival with the increase in gamma dose.

Number of tillers per clump

Analysis of variance revealed non-significant differences among mutation treatments when the buds were primed in water for 12 hrs (Table 3 and 5). The mean number of tillers per clump ranged from 3.60 to 4.30 with an average of 4.10 when the buds were primed in water for 12 hrs where as it ranged from 3.60 to 4.6 when non primed fresh cut buds were sown after irradiation treatment. Among irradiation treatments highest number of tillers per clump was recorded in control (5.15) followed by 10 Gy (4.45) and minimum in 30 Gy (2.45). The variability recorded for this trait is also shown in Fig. 1. Chaudhary (1971) reported non-significant differences for tiller numbers in M_1V_2 generation. These results are in agreement with the results of **Yasmin** *et al* (2011) who induced genetic variation in sugarcane through mutagenesis with doses of gamma rays 10, 20 30 and 40 Gy. They reported that different irradiation doses showed significant impact on average number of tillers, as the dose increased the tillering potential decreased. In the present study no significant differences were found for primed and unprimed treatments.

Number of millable canes per clump

Total number of tillers per clump does not reflect truly to number of millable canes but it is a general observation that a plant having higher number of tillers usually has a high number of millable canes. In this study, analysis of variance exhibited significant differences for this trait among different doses of gamma rays applied to the sugarcane buds (Table 3). The mean number of millable canes per clump ranged from 2.30 to 4.00 with an average of 3.40 in the primed buds but it ranged from 2.30 to 4.00 in unprimed treatment with an average of 3.20 (Table 5). Mean number of millable canes was observed to be highest in control (4.00) followed by 10 Gy (3.60) and minimum in 30 Gy (2.30) having similar trend (Fig. 2) same as observed for number of tillers per clump (i.e. tended to drop with an increase in dosage). Various factors such as inhibition of auxin synthesis (Gurdon 1954), production of diffusible

growth retarding substances (Mackey 1951) and inhibition of DNA synthesis (Gaul 1970) have all been reported to be able to effect reduced growth in irradiated plants or seeds.

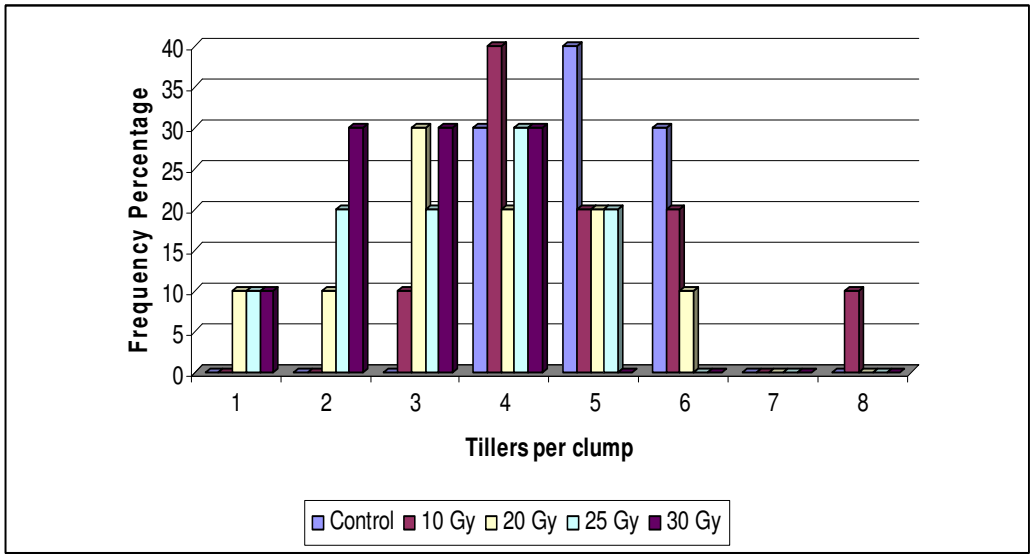


Fig. 1 Percent clumps in M_1V_2 generation showing variation in number of tillers at different doses γ-irradiation

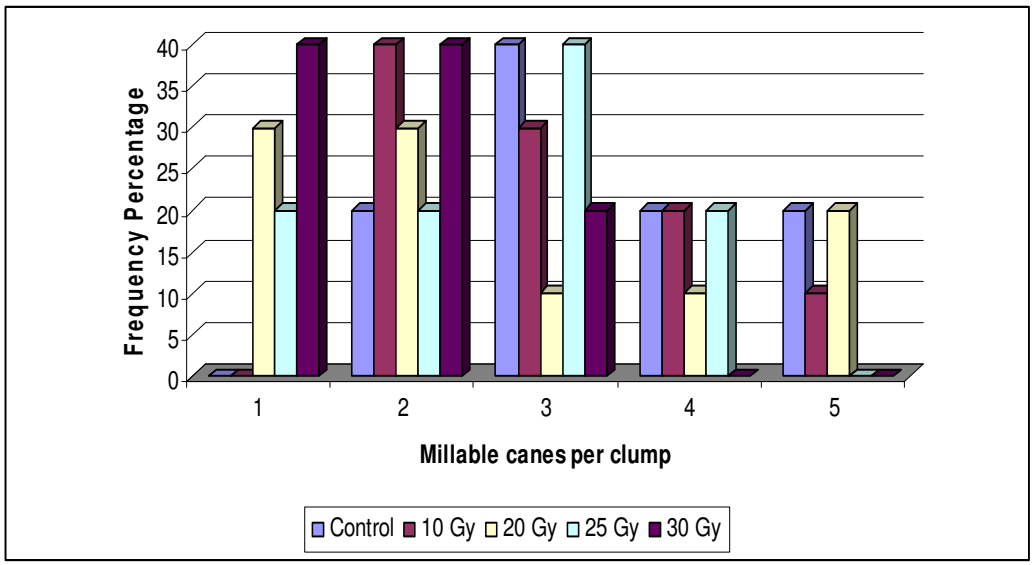


Fig. 2 Percent clumps in M_1V_2 generation showing variation in number of millable canes at different doses γ-irradiation

Cane height (cm)

Significant differences among the different mutation treatments were observed from the analysis of variance (Table 3). Average cane height among different irradiation treatments ranged from 166.60 to 182.70 cm in water primed buds (Table 5). The mean height was recorded to be 171.2. However, in unprimed treatment height ranged from 157.40 to 185.80 cm for different mutation treatments. Highest cane height of 185.80 cm was observed in 30 Gy as shown in Fig. 3. Khan et *al* (2009) have also reported higher cane height in gamma ray

irradiated clones of sugarcane than control. They observed significant differences among treatment for this trait and recorded maximum plant height for gamma ray treatment of 20 Gy. They also found maximum cane height in clones of sugarcane variety NIA-2004 when mutagenic treatment was applied at the rate of 30 Gy. In present study, the dose of 30 Gy showed stimulating and enhancing effect on plat height which is in complete agreement with earlier findings of Khan *et al* (2007) who reported the genetic variability in sugarcane induced through mutation breeding.

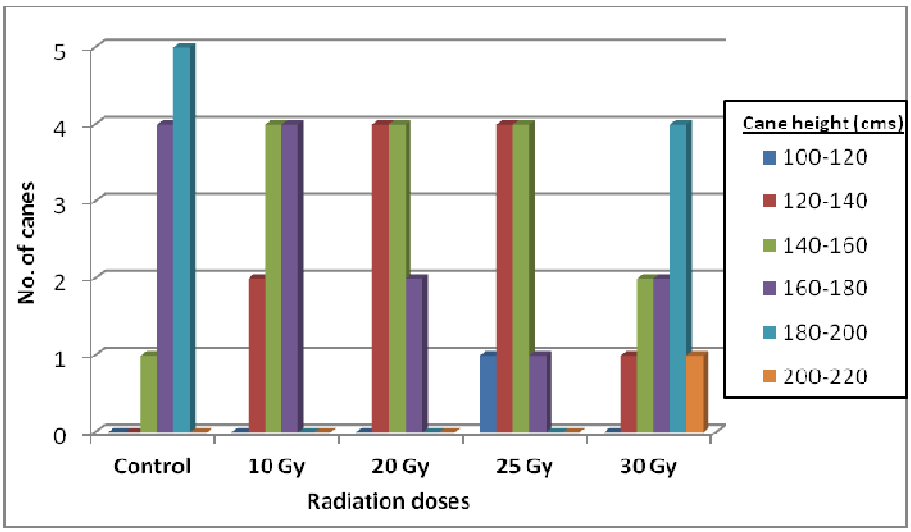


Fig. 3 Variability for cane height in M_1V_2 generation at different doses of γ-irradiation

Cane girth (cm)

There are no significant differences for cane girth among the different mutation treatments in sugarcane buds evaluated during spring 2013 (Table 3). Average cane girth in different mutation treatments ranged from 2.10 to 2.30 cm when the buds were primed for 12 hrs in water and girth ranged from 2.10 to 2.40 cm in non primed buds (Table 5). There were no significant differences found for priming and non priming of sugarcane buds in water. Highest cane thickness of 2.40 cm (Fig. 4) was recorded in 30 Gy. Earlier these types of results were obtained by Khan *et al* (2007). In this study, higher mutation did not reflect significant difference in cane thickness in mutated population for doses of 20, 25 and 30 Gy.

HR Brix (%)

For refractometer brix the results were significantly different for different doses of gamma irradiation in sugarcane buds treated to induce genetic variability. Refractometer brix in different mutation treatments used in present study ranged from 21.00 to 23.10 percent in primed buds with an average of 21.92 and which is at par with the values of brix in non primed buds (Table 5). These results are also similar to the findings of Kwon- Ndung *et al* (2000). Maximum refractometer brix (23.10 %) was recorded for control followed by 10 Gy (22.10). Minimum refractometer brix (21.50) was recorded in population treated with gamma

ray at the rate of 30 Gy (Fig. 5). Similar response was reported by Khan et *al* (2009 and 2007) who recorded low sucrose (%) and CCS (%) in 20 Gy and high in 40 Gy and 50 Gy, respectively.

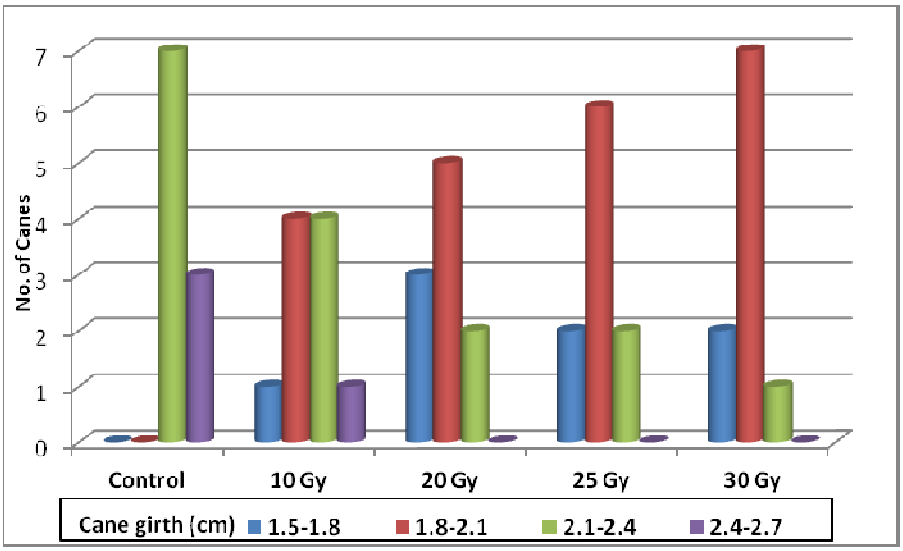


Fig. 4 Variability for cane girth in M_1V_2 generation at different doses of γ-irradiation

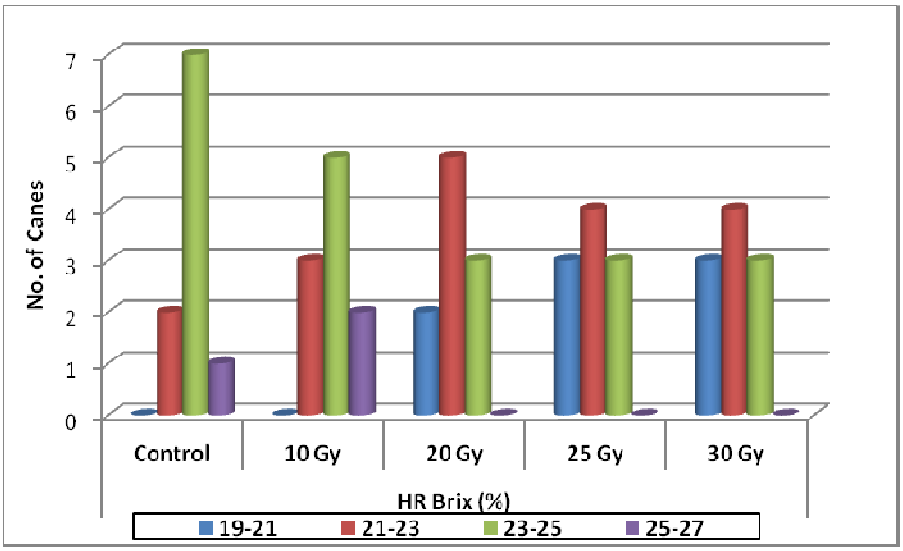


Fig. 5 Variability for HR Brix in M_1V_2 generation at different doses of γ-irradiation

Leaf length (cm)

Significant differences among mutation treatments were observed for leaf length in the study for M_1V_2 sub clones during spring 2013 (Table 3). Mean value of leaf length for different treatments varied from 108.80 to 119.10 cm with an average of 114.30 cm in primed and 100.00 to 118.10 cm with an average of 111.56 cm in non primed treatment (Table 5). Maximum leaf length (119.1 cm) was recorded in control followed by 10 Gy**.** These results are in accordance with the findings of Hegde (2006) who studied effect of gamma irradiation dose on the vegetative characters in Turmeric (*Curcuma longa* L.) and reported that lower

irradiation dosages have enhancing effect on leaf length. In this study, minimum dose i.e. 10Gy showed the maximum leaf length followed by the control. Singh *et al* (1999) also observed similar results using three doses of gamma irradiation in sugarcane.

Leaf width (cm)

Analysis of variance exhibited non significant differences among the different mutation treatments for leaf width in this study for M_1V_2 sub clones (Table 3). Average value of leaf width ranged from 3.30 to 4.20 cm among the different mutagenic treatments in the irradiated material when priming was done prior to irradiation while it ranged from 3.30 to 4.10 cm in the unprimed irradiated buds (Table 5). Maximum leaf width was observed in 10 Gy dose (4.2 cm) which is at par with control (4.00 cm). These results were according to the results found by Hegde (2006) in turmeric cultivar and also by Singh *et al* (1999).

Cane weight (g)

Cane weight is the ultimate trait in sugar cane breeding programme. Due to low genetic variability in this crop exerted efforts are being made to enhance variability using different approaches. Perusal of data appended in Table 3 and Fig. 6 revealed that significant differences exist for this important trait among different mutagenic treatments. Single cane weight in M_1V_2 irradiated sub clones of variety Co J 64 ranged from 706.66 to 975.66 g in primed buds while the corresponding values for unprimed buds were 710.00 to 961.66 g (Table 5). Among various mutagenic treatments maximum single cane weight was recorded for control closely followed by 10 Gy and 20 Gy. The higher doses have retarding effect on this trait. Significant high primary stalk weight for lower gamma irradiation dose (20 Gy) as compare to 40 Gy has also been reported by Khan *et al* (2007) in sugarcane which is in complete agreement with present results.

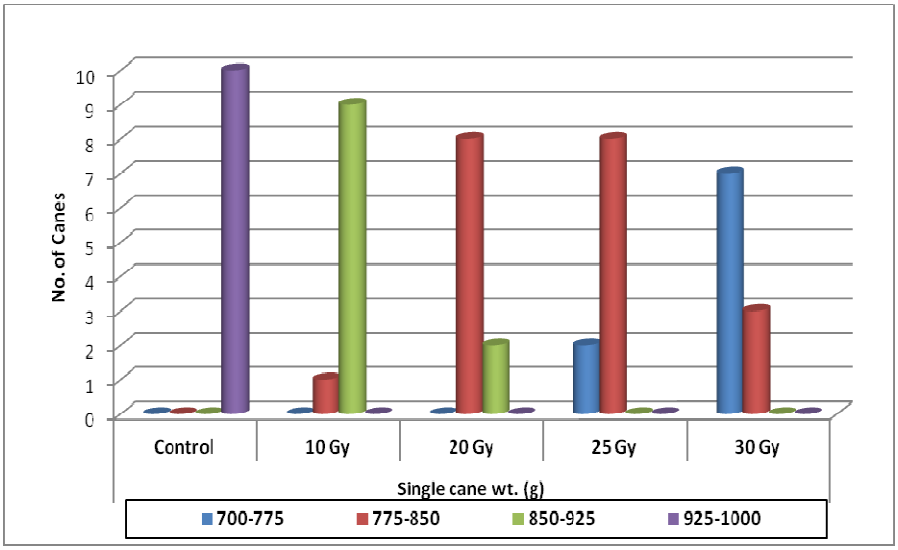
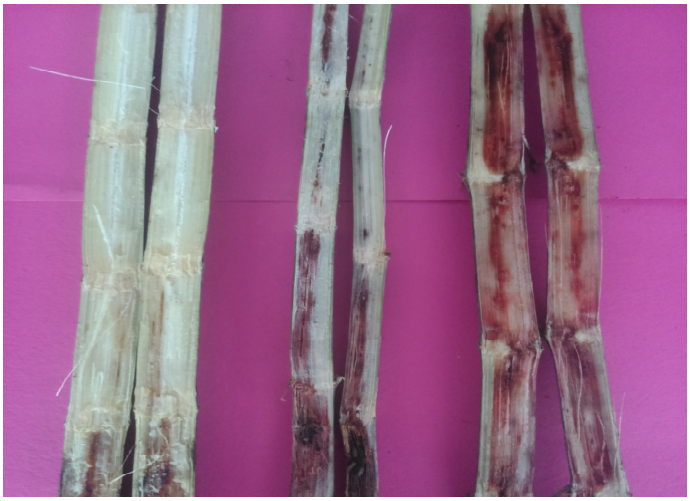


Fig. 6 Variability for single cane weight in M_1V_2 generation at different doses of γ-irradiation



Irradiated sub-clones (M_1V_2 generation) showing differential response to red rot disease (artificial inoculation).

PLATE 4

Evaluations of irradiated sub clones against red rot disease

The experimental material generated through different mutation treatments in present study was planted at PAU, RRS Kapurthala during spring 2013 (M_1V_2 generation) was evaluated for red rot resistance. Pathotypes Cf 08 and Cf 09 were multiplied on oat meal agar medium in Petri dishes at 25±1ºC. The spore suspensions with concentration of $2X10^4$ conidia ml^{-1} was maintained and inoculated. Ten canes per treatment were inoculated by a suspension of Cf 08 and Cf 09 pathotypes separately by artificial inoculation under field conditions using plug method (Srinivasan and Bhatt 1961). Disease data were recorded after 60 days of inoculation after splitting the canes longitudinally (Plate 4).

Observations were recorded on the number of internodes transgressed by the pathogen. The canes were rated 0-9 as per scale of Srinivasan and Bhatt (1961). The disease score of irradiated subclones recorded in present study is reported in Table 6.

Table 6 Disease reaction of irradiated sub clones of Co J64 against two red rot pathotype in M_1V_2 generation

Gamma ray treatment (Gy)	Disease assessment score		Reaction	Per cent sub-clones
	Cf 08	Cf 09		
0	7.30	8.40	HS	100
10	3.20	3.30	MR	20
20	3.30	3.30	MR	20
25	3.40	4.60	MS	90
30	3.40	3.20	MR	10

MR- Moderately resistant, MS-Moderately susceptible

Perusal of table 6 revealed that control population was highly susceptible to red rot disease. Among various mutagenic treatments the 10 and 20 Gy irradiated population, 20% canes obtained were found to be moderately resistant. But in 25 Gy irradiated population, 90% canes were moderately susceptible whereas in 30 Gy population moderately resistant canes obtained were 10%.

Present study showed that by using gamma-irradiation at lower doses of 10 and 20 Gy useful genetic variability could be induced in sugarcane for economic traits like cane height, HR Brix, single cane weight and disease score.

CHAPTER - V

SUMMARY

Sugarcane is a highly heterozygous polyploid species (2n=80 to 205). Modern sugarcane varieties are complex hybrids arising primarily from *S. officinarum, S barberi, S. sinese* hybridized with *S. spontaneum.* Natural or induced genetic diversity is a vital component of a plant breeding program. In sugarcane, molecular diversity studies have revealed that the modern commercial cultivars have developed from a limited genetic base and were derived from only 20 *S. officinarum* clones and less than ten *S. spontaneum* derivatives. Development of a sugarcane variety with stable performance has been a challenging task for sugarcane breeders. Therefore, the present investigation entitled 'Irradiation induced mutagenesis in sugarcane' was undertaken to create genetic variability in Co J 64 variety of sugarcane which is an early maturing, high yielding and high sugar but susceptible to red rot disease. Single bud setts (1200) of Co J 64 were irradiated with gamma rays in gamma chamber located at Department of Horticulture, Punjab Agricultural University, Ludhiana for different time intervals *viz.* 1min 17 sec, 2 min 34 sec, 3 min 13 sec and 3 min 52 sec for 10, 20, 25 and 30 Gy, respectively. The material generated was used to grow first (M_1V_1) generation by following two priming methods. The experimental plant material M_1V_1 generation obtained from different treatments was planted in the Sugarcane experimental area of Punjab Agricultural University, U.S.F, Ladhowal during spring 2012 and second generation (M_1V_2) at PAU, Regional Research Station Kapurthala during spring 2013 for its evaluation under field condition. Recommended package of practices were followed to raise the healthy crop stand. The data were recorded on 10 agro-morphological and quality traits in addition to disease score for red rot against two pathotypes.

Analysis of variance revealed non-significant differences among priming treatment for all the traits studied in M_1V_1 generation. However, mutation treatments revealed significant differences for germination, number of tiller/ clump, number of millable cane/ clump, cane height, HR brix, Leaf length and single cane weight while non significant for cane girth and leaf width of sugarcane in M_1V_1 sub clones of Co J 64 variety grown during spring 2012. Similarly, in M_1V_2 sub clones grown during spring 2013 at RRS Kapurthala significant differences exist for many traits except number of tiller / clump, number of millable cane/ clump and cane girth indicating the potential of mutagenic treatments for creating genetic variability for different traits in sugarcane crop.

The per cent germination of irradiated buds after 45 days of planting were recorded for different treatments revealed significant difference for this trait among mutation treatments and treatment x priming interactions. The germination percentage of irradiated buds ranged from 27.50 to 43.16 per cent in M_1V_1 generation grown during spring 2012. The

highest germination was (43.16%) for control closely followed by mutation dose of 10 Gy. Germination percentage decreased with increase in gamma rays dose the lowest being for 30 Gy. The number of plantlets survival after 120 days under field conditions showed that there were significant differences among treatments for per cent survival rate under both primed and non-primed conditions. The highest per cent survival was recorded for control 0 Gy (86 %) and minimum for 30 Gy (47.66%). Of the different treatment combinations (TxP) interactions survival percentage ranged from 43.33% to 90.00%.

The mean number of tillers per clump ranged from 2.60 to 5.30 with an average of 3.80 when the buds were primed in water for 12 hrs where as it ranged from 2.30 to 5.00 when non primed fresh cut buds were sown after irradiation treatment. Among irradiation treatments highest number of tillers per clump was recorded in control (5.15) followed by 10 Gy (4.45) and minimum in 30 Gy (2.45). The mean number of millable canes per clump ranged from 2.30 to 4.00 with an average of 3.40 in the primed buds but it ranged from 2.30 to 4.00 in unprimed treatment with an average of 3.20. Mean number of millable canes was observed to be highest in control (4.00) followed by 10 Gy (3.60) and minimum in 30 Gy (2.30) having similar trend same as observed for number of tillers per clump. Average cane height among different irradiation treatments ranged from 166.60 to 182.70 cm in water primed buds. The mean height was recorded to be 171.2. However, in unprimed treatment height ranged from 157.40 to 185.80 cm for different mutation treatments. Highest cane height of 185.80 cm was observed in 30 Gy. Refractometer brix in different mutation treatments used in present study ranged from 22.20 to 23.80 percent in primed buds with an average of 22.80 and which is at par with the values of brix in non primed buds. Maximum refractometer brix (23.80%) was recorded for 30 Gy treated population followed by control (23.40). Minimum refractometer brix (22.20) was recorded in population treated with gamma ray at the rate of 20 Gy. Mean value of leaf length for different treatments varied from 106.30 to 114.40 cm with an average of 111.40 cm in primed and 105.70 to 114.80 cm with an average of 111.00 cm in non primed treatment. Maximum leaf length (114.80 cm) was recorded in 10 Gy treated population followed by control**.**

Cane weight is an important trait in sugar cane breeding programme. Due to low genetic variability in this crop exerted efforts are being made to enhance variability using different approaches. Single cane weight in irradiated sub clones of variety Co J 64 ranged from 726.66 to 961.66 g in primed buds while the corresponding values for unprimed buds were 720.00 to 931.66 g. Among various mutagenic treatments maximum single cane weight was recorded for control closely followed by 10 Gy and 20 Gy. The higher doses have retarding effect on this trait.

Reaction of irradiated subclones to two red rot pathotypes Cf 08 and Cf 09 under artificial inoculation by plug method revealed moderate resistance for all the mutation

treatments with disease score ranging from 3.20-3.40.

From the present study it is inferred that genetic variability could be induced in sugarcane using gamma rays for traits like germination, cane height, leaf length, HR brix, single cane weight and disease score. The lower doses of gamma ray (10 and 20 Gy) were more effective to induce variation while higher doses have detrimental effect on different traits especially germination and survival of sub-clones. The subclones identified for high single cane weight coupled with higher HR brix, cane girth and length and low disease score need to be further evaluated in M_1V_3 for their consistency in trait manifestation and future use in breeding programme.

REFERENCES

Abraham M and Radhakrishnan V V (2009) Induced Mutations in Coleus (*Solenostemon rotundifolius*) an Under-Utilized Medicinal Tuber. In: Shu Q Y (ed) *Induced plant mutations in genomic era.* Pp 283-85. FAO United Nations, Rome.

Ahloowalia B S, Maluszynski M and Nichterlein K (2004) Global impact of mutation derived varieties. *Euphytica* **135**: 187 – 204.

Ali A, Naz S, Alam S and Iqbal J (2007) *In vitro* induced mutation for screening of red rot (*Colletotrichum falcatum*) resistance in sugarcane (*Saccharum officinarum*). *Pak J Bot* **39**: 1979-94.

Arruda P (2011) Perspective of the sugarcane industry in Brazil. *Tropical Pl Biol* **4** : 3-8.

Auti S G and Apparao B J (2009) Induced mutagenesis in mungbean (*Vigna radiata* (L.). In: Shu Q Y (ed) *Induced Plant Mutations in the Genomics Era.* Pp 97-100 FAO United Nations, Rome.

Banerji B K and Datta S K (1991) Induction of somatic mutations in chrysanthemum cultivar 'Anupam'. *J Nucl Agric Biol* **19** : 252 – 56.

Bari C (1974) Induction of somatic mutations for disease resistance in sugarcane. *Proc Induced mutations for disease resistance in crop plants.* Pp 149. IAEA Vienna, Austria.

Bhagyalaxmi K V (1975) *Studies on induction of mutations in sugarcane*. M Sc Thesis, Bhavan's College Andheri, Bombay, India.

Breanx R D (1975) Radiosensitivity and selection for mosaic resistant variety in sugarcane. *Int Sac Sugar Cane Tech* **4**: 97-100.

Broertjes C And Van H A M (1988) *Applied mutation breeding for vegetatively propagated crops.* Elsevier, Amsterdam.

Brunner H (1995) Radiation induced mutations for plant selection. *Appl Radiat and Isotopes* **46**: 589-594.

Burner D M and Legendre (1997) Agronomic performance and transmission of the multiple-bud mutation of sugarcane. *Crop Sci* **38** : 928-32.

Busey P (1979) Gamma ray dosage and mutation breeding in *S. augustinegrass*. *Crop Sci* **20** : 181-84.

Chaudhary H K (1971) *Elementary principles of plant Breeding*. Oxford and IBL publishing co ,New Delhi.

Cheema H S and Singh B (1990) A user's manual to CPCS-1. A computer Programme Package for the Analysis of Commonly Used Experimental Designs, pp-1, PAU, Ludhiana.

Chen C, Lu S, Chen Y, Wang Z, Niu Y and Gou Z (2009) A Gamma- ray-induced dwarf mutant from seeded bermudagrass and its physiological responses to drought stress. *J Am Soc Hort Sci* **134** : 22-30.

Chezhiyan N and Shanmugasundaram K A (2000) BSR 2- A promising turmeric variety from Tamil Nadu. *Indian J Arecanut, Spices and Medicinal plants* **2:** 24-26.

Chopra V L (2005) Mutagenesis : investigating the process and processing the outcome for crop improvement. *Curr Sci* **89**: 353-59.

Das P K, Dube S and Dey A K (1978) New dahlias through irradiation. *Indian Hort* **23**: 19 – 21.

Datta S K and Banerji B K (1990) Los Banos variegate – a new double bracted chlorophyll variegated bougainvillea induced by gamma rays. *J Nucl Agric Biol.* **19**:134 –36.

Dermodjo S (1977) Induction of mosaic disease resistance in sugarcane by gamma ray irradiation. *Int Soc Sugar Cane Tech Sug Br Newsl* **39**: 4-7.

*Ehrenberg L (1960) Induced mutation in plants mechanism and principles. *Genet Ag* **12**: 364-89.

*Favret E A (1960) Induced mutations for resistance to diseases. *Genet Ag* **13**: 1-2.

Fisher, R A (1954) *Statistical Methods for Research Workers*. Oliver and Boyd, Edinburg.

*Freisleben and Lein (1944) [Moglichkeiten und praktische durchtuhrung der mutationszuchtung. *Kuhn Ashiv]* **60**: 211-22. (In German)

Gaul H (1970) Mutagen affects observably in the first generation. Plant injury and lethalty. Cytogenetical effects. Sterility. Manual on mutation breeding. 85-99(Technical Report Series No. 119. IAEA, Vienna, Austria.

Giridharan M P and Balakrishnan S (1992) Gamma ray induced variability in vegetative and floral characters of ginger. *Indian Cocoa, Arecanut and Spices J* **15** : 68 – 72.

Girija M and Dhanavel D (2009) Mutagenic effectiveness and efficiency of gamma rays ethyl methane sulphonate and their combined treatments in Cowpea (*Vigna unguiculata* L. Walp). *Global J Mol Sci* **4** : 68-75.

Gnanamurthy S, Mariyammal S, Dhanavel D and Bharathi T (2012) Effect of gamma rays on yield and yield component characters R3 generation in cowpea (*Vigna unguiculata* (L.) Walp). *Int J Res in Pl Sci* **2**: 39-42.

Gupta M N, Laxmi V, Dixit B S and Srivastava S N (1982) Gamma ray induced variability in *Costus speciosus. Prog Hort* **14** : 193 – 97.

Gurdon S A (1954) Occurrence, formation and inactivation of auxins. *Annl Rev Pl Physiol* **5**: 341-78.

Haq M S, Maniruzzaman A F M and Alt S M (1970) Evolution of high yielding disease resistant strains of sugarcane through gamma irradiation. *Proc 21st-22nd Pak Sci Conf.* Association advancement Sci, Lahore, Pak.

Haq M S, Rehman M M, Mia M M and Ahmed H U (1974) Disease resistance of some mutants induced by gamma rays. *Proc Induced mutations for disease resistance in crop plants*. Pp 150. IAEA, Vienna, Austria.

Haw M S, Tin M M and Myant T (1974) The prospect of breeding red-rot resistance sugarcane varieties through induced mutations in Burma. *Proc Induced mutations for disease resistance in crop plants.* Pp 151. IAEA, Vienna, Austria.

Hedge R V (2006) *Studies on Induced Mutagenesis and in vitro regeneration in Turmeric (Curcuma longa* L.*).* Ph D Dissertation, University of Agricultural Sciences, Dharwad.

Hemalatha K (1998) *Induction of mutation in carnation (Dianthus caryophyllus* L.) *through gamma rays and ethyl methane sulphonate.* Ph.D. Dissertation, University of Agricultural Sciences, Bangalore, India.

Hrishi N and Marimuthammal (1968) Studies on mutagenesis in sugarcane. *Pl Sci* **68**:131-42.

Ibrahim R, Hamzah A, Jan Jam Z, Bahagia M and M Joyo (2009) Gamma Irradiation-Induced Mutation for the improvement of josapine pineapple against bacterial heart rot disease and improved fruit quality. In: Shu Q Y (ed) *Induced plant mutations in genomic era.* Pp 283-85. FAO United Nations, Rome.

Jagathesan D (1977) Induction and Isolation of mutants in sugarcane. *Mutat Breed Newsl* **9**: 5-6.

Jagathesan D (1982) Improvement of sugarcane through induced mutations. *Panel Proc Series* 139-53.

Jagathesan D and Ratnam R (1970) A vigorous mutant of sugarcane *(Saccharum* spp.) clone Co 527. *Theor Appl Genet* **51**: 311-13.

Jagathesan D and T V Sreenivasan (1970) Induced mutations in sugarcane. *Indian J Agric Sci* **40:** 165-72.

Jagathesan D, Balasundaran N and Alexander K C (1974) Induced mutations for disease resistance in sugarcane. *Proc Induced Mutations for Disease Resistance in Crop Plants.* Pp. 151. IAEA, Vienna, Austria.

James G (2004) *Sugarcane*. Pp 1-214. Blackwell Publishing, Oxford.

Jayachandran B K and Kumar M N (1992) Effect of gamma ray irradiation on ginger. *South Indian Hort* **40** : 283 – 88.

Kaur A and Gosal S S (2009) Optimization of gamma radiation dose for induction of genetic variation in sugarcane *(Saccharum* spp) callus and regenerated shoot cultures. *J Pl Biochem Biot* **18**:117-20.

Kenganal M, Hanchinal R R and Nadaf H L (2008) Ethyl methane sulphonate (EMS) induced mutation and selection for salt tolerance in sugarcane in vitro. *Indian J Pl Physiol* **13**: 405-10.

Khairwal I S, Singh S, Paroda R S and Yaneja (1984) Induced mutations in sugarcane- effects of physical and chemical mutagens on commercial cane sugar and other quality traits. *Indian Natn Soc Acad* **50** : 505-11.

Khan I A , Bibi S, Yasmin S, Khatri A, Nighat S And Afghan (2010) genetic variability in mutated population of sugarcane clone Nia-98 through molecular markers. *Pak J Bot* **42**: 605-14.

Khan I A, Dahot M U, Nighat S, Yasmin S, Bibi S, Raza S and Khatri A (2009) Genetic variability in sugarcane plantlets developed through in vitro mutagenesis. *Pak J Bot* **41**: 153-66.

Khan I A, Umar D M and Abdullah K (2007) Study of genetic variability in sugarcane induced through mutation breeding. *Pak J Bot* **39**: 1489-1501.

Khan M A W, Veeraswamy R (1974) Mutations induced in red gram (*Cajanus cajan* L Millsp) by gamma radiation and EMS. *Radiat Bot* **14**: 237-42.

Khan S J, Khan H U, Khan D R, Malik M I and Zafar Y (2000) Development of sugarcane mutants through *in vitro* mutagenesis. *Pak J Biol Sci* **3**: 1123-25.

Krishnamurthi M and Tlaskal J (1974) Fiji disease resistant *Saccharum* var. Pindar subclones from tissue culture. *Int Soc of Sugar Cane Technol* **15**: 130-36.

Kwon-Ndung E H and Ifenkwe O P (2000) Mutational Studies on Sugarcane Using Gamma Irradiation. *Sugar Tech* **2** : 29-35.

Lacey C N D and Campbell A I (1977) Character changes in induced mutants of Bramley's seedling apple. *Acta Hortic* **75**: 51 – 62.

Lapins K O, Bailey P H and Hough L F (1969) Effect of gamma rays on apple and peach buds at different stages of development, survival, growth and mutation frequencies. *Radiat Bot* **9**: 379 – 89.

*Mackey J (1951) Neutron and X-ray experiment in a barley. *Hereditas* **37**:421-64

Majid M A, Shamsuzzaman K M, Howlider M A R and Islam M M (2001) Development of sugarcane mutants with resistance to red rot, water-logging and delayed or non-flowering through induced mutations. *Proc Final Res Coord Meet.* Pp 31-43 IAEA, Vienna, Austria.

Malamug J J, Yazawa S and Asahira T (1974) Morphological variants induced from shoot tips of taro (*Colocasia esculenta* L Schott) treated with gamma radiation. *Scientia Hort* **58**: 105-13.

Malhotra K C and Vijaybhanu B (1969) Spontaneous somatic mutations in BO Canes. *Sci cult* **35**:100-03.

Maria I O, Victor G, Luis R, Novisel V, Hofte M and Jimenez E (2012) Selection and characterisation of sugarcane mutants with improved resistance to brown rust obtained by induced mutation. *Crop Pasture Sci* **62**: 1037-44.

McCollum D G (1977) Growth response of potato tuber eyes to irradiation with X-rays and fast neutrons. *Am J Potato Res* **54** : 593-602.

Mei M, Deng H, Lu Y, Zhuang C, Liu Z, Qiu Q, Qiu Y and Yang T C (1994) Mutagenic effects of heavy ion radiation in plants. *Adv Space Res* **10**: 363-72.

Miedema P (1973) The use of adventitious buds to prevent chimerism in mutation breeding of potato. *Euphytica* **22** : 209-18.

Moh C C and Alan J J (1974) Radio sensitivity of some tropical plant species. *Turrialba* **24**: 156-59.

Mohanty D C and Panda B S (1988) Variability in rhizome of *Zingeber officinale* by mutagenesis and comparison of gamma radiation and ethyl methane sulphonate for crop improvement. *Curr Sci* **89**: 353 –59.

Mustafakhan A and Zakir A (1970) Improvement of sugarcane through radiation. *West Pak J Agric Res* **8**: 1-6.

Nair M K (1973) Note on induced red-rot resistant mutant of Co 997 variety of sugarcane. *Indian J Agric Res* **43**: 323-24.

Neto A T, Domingues E T, Mendes B M J and Ando A (1989) *In vivo* methods for the induction of mutations in 'Maca' banana improvement programmes. *Revista Brasileira de Genetica* **12** : 871 – 79.

Novak F J and Brunner H (1992) *Plant Breeding Induced Mutation Technology for Crop Improvement:* Tech Bull 4, Pp25-33. IAEA, Vienna, Austria.

Nwachakwu E C, Ene L S O and Mbanaso E N A (1994) Radiation sensitivity of two ginger varieties (*Zingiber officinale* Rose) to gamma irradiation. *Tropenlandwirt* **95** : 99 – 103.

Nwachukwu E C, Ene L S O, Mbanaso E N A and Ago F M O (1990) Increasing variability in yams by induction of mutation using gamma rays. Annual Report . National root crop research institute Amidic, Omaha Nigeria.

Nwachukwu E C, Mbanaso E N A and Nwosu K I (2009) The development of new genotypes of the white yam by mutation induction using yam mini-tubers. In: Shu Q Y (ed) *Induced Plant Mutations in the Genomics Era*. Pp 309-12. FAO United Nations, Rome.

Panje R R and Prasad P R J (1959) The effects of ionizing radiations on sugarcane. *Curr Sci* **28**: 183-226.

Patade V Y and Suprasanna (2008) Radiation induced *in vitro* mutagenesis for sugarcane improvement. *Sugar Tech* **10**: 14-19.

Poehlman J M and Sleper D A (1995) *Breeding Field Crops*. Pp.1-278. Panima Publishing Corporation, New Delhi.

Powell B J, Burton W G and Young R J (1973) mutations induced in vegetatively propagated turf bermudagrass by gamma radiation. *Crop Sci* **14**: 327-30.

Premsekar S and Appadurai A (1981) Effect of doses of gamma rays and ethyl methane sulphonate on the germination and survival of induced mutations in pigeon pea. *Indian J Agric Sci* **51**:381-86.

Price S and Warner J N (1959) The possible use of induced mutations for sugarcane improvement. *Proc Int Soc Sugarcane Technol 10th Cong*. Pp 782-91.

Raghuvanshi S S and Singh Anil K (1979) Gamma -ray induced mutations and autotetraploid perennial *Portulaca grandiflora,* Hook. *Indian J Hort* **36**: 84-87.

Rangaswamy S R (1986) Applied mutation research in field crops at Tamil Nadu Agricultural University. *Mutat Breed Newsl* **28:**13-14.

Rao J T, Srinivasan K V and Alexander K C (1966) A red-rot resistant mutant of sugarcane induced by gamma irradiation. *Indian Acad Sci* **64**: 224-25.

Rao B and Reddi V R (1975) A radiation-induced highly productive mutant in sorghum. *Radiat Bot* **15**: 29-32.

Rao B V (1954) A brief review of work done on the use of X-rays in sugarcane breeding. *Proc 2nd Sugarcane Res Dev Workers Conf* Pp 120-27. Jullundur, Mysore.

Rao D V R (1999) Effect of gamma irradiation on growth, yield and quality of turmeric. *Adv Hortic For* **6**:107-10.

Rao H K S, Sachan R S and Singh R S (1977) Mutation breeding and its implication in the sugar industry. *Proc Indian Sug Technol Assoc 6th Joint Congress*. Pp. 163-66. STAI, DSTA and SISTA, Madras.

Rao P S (1974) Mutation breeding for non flowering in sugarcane. *Mutat Breed Newsl* **3**: 9.

Roach B T (1974) Quantitative effects of radiation induced mutation in sugarcane. *South Af Sugar J* **58**: 11.

Roy S, Vardi A and Elhanati A (1990) Seedless induced mutant in highly seeded lemon. *Mutat Breed Newsl* **36**: 11.

Sadat S and Hoveize M S (2012) Mutation induction using ethyl methanesulfonate (EMS) in regenerated plantlets of two varieties of sugarcane CP48-103 and CP57-614. *Af J Agric Res* **7**: 1282-88.

Selim A K A, Marwan M A, Tawab A F M and Rashedi H A (1980) *Effect of gamma irradiation on two sugarcane varieties.* Res Bull 13. Pp 21. Faculty of Agriculture, Ain Shams University.

Sen A and Alikamanoglu S (2012) Analysis of drought-tolerant sugar beet (*Beta vulgaris* L.) mutants induced with gamma radiation using SDS-PAGE and ISSR markers. *Mutat Res Fund Mol Mech Mutagenesis* **738**: 38-44.

Shah S S (1972) Role of sugarcane breeding institute in improvement of agriculture in India. *Indian Farming* **22**: 38-44.

Shankaranarayanan P and Bubu C N (1970) Effect of chronic gamma irradiation in sugarcane varieties. *Indian J Heredity* **2**: 97-102.

Sharma D K and Majumdar P K (1988) Induction of variability in mango through physical and chemical mutagens. *Acta Hortic* **231** : 112 – 16.

Siddiqui S H and Javed M A (1982) Mutation breeding in sugarcane (*Saccharum* sp. Hybrid) by gamma irradiation of cuttings and tissue culture. *Proc Induced Mutations in Vegetatively Propagated Plants II.* Pp. 155-166. IAEA, Vienna.

Siddiqui S H, Mujeeb K A and Keerio G R (1975) Gamma irradiation effect on sugarcane *(Saccharum* spp.) clone Co 547. *Environ Exp Bot* **16**: 65-68.

Singh (1970) Chronic gamma irradiation induced resistance to red rot disease in sugarcane variety Co 997. *Sugar Cane Path Newsl* **5**: 24.

Singh K P, Singh B, Raghava S P S and Kalia C S (2000) Induced flower color mutations in carnation through *in vitro* application of chemical mutagen. *Indian J Genet* **60**: 535-39.

Singh R K, Chandra P, Singh D N, Singh R G and Singh S B (1999) Morphologically distinct mutants in sugarcane and their protein profile. *Sugar tech* **1**: 93-97.

Singh R N and Iyer C P A (1972) Standardization of dosimetry and technique for mutations in pineapple. *Indian J Hort* **29**: 342 – 43.

Smith H H (1958) Radiation in the production of useful mutations. *Bot Rev* **24**: 1-24.

Snedecor G W and Cochran W G (1967*) Statistical Methods*.6th edition. Pp 593. Oxford and IBL Publication Co Calcutta.

Song L I, Jian-hua Y, Kun-xing Y U, Hong-jian L, Jian W E I, Min L L, Ming D, Fing T, Man-man L (2011) Mutation breeding of new sugarcane variety GUIFU 98-296. *J Nucl Agric Sci* **24**: 1177-81.

Sparrow A H, Underbrink A G and Sparrow R C (1967) Chromosomes and cellular radiosensitivity relationship of dose to chromosome volume and complexity in seventy nine different organisms. *Radiat Res* **32**: 915 – 45.

Sreenivasan T V, Ahloowalia B S and Heinz D J (1987) In: Heinz D J (ed) *Sugarcane Improvement through Breeding*. Pp 211-53. Elsevier, Amsterdam.

Srinivasan K V and Bhat N R (1961) Red rot of Sugarcane: Criteria for breeding resistance. *J Indian Bot Soc* **40**: 566-77.

Srivastava B L, Bhat S R, Pandey S, Tripathi B S and SaxenaV K (1986) Plantation breeding for red rot resistance in sugarcane. *Sugarcane* **5**: 13-15.

Suprasanna P, Patade V Y, Vaidya1 E R and Patil V D (2009) Radiation induced *in vitro* mutagenesis, selection for salt tolerance and characterization in sugarcane. *Induced Plant Mutations in the Genomics Era*. Pp145-147. FAO United Nations, Rome.

Suprasanna P, Sidha M, Bapat V A (2009) Integrated approaches of mutagenesis and *in vitro* selection for crop improvement. In: Kumar A and Shekhawat N S (ed) *Plant Tissue Culture, Molecular Markers And Their Role In Crop Productivity*. Pp 73-92. IK International Publishers, New Delhi.

Teruo N, Yukiko 1, Yoshie M, Yoshinori M and Hideyoshi T (2001) Induction of morphologically changed petals from mutagen-treated apical buds of rose and plant regeneration from varied petal-derived calli. *Pl Biot* **18** : 233-36.

Tysdal H M (1956) Promising new procedures in sugarcane breeding. IN: *Proc* 1" *Cong Int Soc Sugar Cane Technol* pp. 618-31.

Vasudevan K N, Nair S G, Jos J S and Magoon M L (1967) Radiation induce mutations in cassava. *Indian J Hort* **24**: 95-98.

Vijailakshmi U and Rao J T (1960) Effects of gamma rays on germination and growth in some specie and hybrids of *Saccharum. Curr Sci* **29**: 397-98.

Wang Y, Wang F, Zhai H and Liu Q (2007) Production of a useful mutant by chronic irradiation in sweet potato. *Scientia Hort* **111**: 173-78.

Yasmin S, Khan I A, Khatri A, Seema N, Siddiqui M. Aquil and Bibi S (2011) Plant regeneration from irradiated embryogenic callus of sugarcane. *Pak J Bot* **43** : 2423-26.

Zambrano A Y, Demey J R, Fuchs M, Gonzalez V, Rea R, De S O and Gutierrez Z (2003) Selection of sugarcane plants resistant to SCMV. *Pl Sci* **165** : 221-25.

Zykov K I and Klimenko Z K (1989) Prospects of shortening the time to breed new garden rose cultivars. *Bot Saad* **108** : 127 –37.

***Original not seen**

VITA

Name of the student	**:**	Mandeep Kaur
Father's name	**:**	Sh. Jai Bhagwan Dass
Mother's name	**:**	Smt. Kamla Devi
Nationality	**:**	Indian
Date of birth	**:**	17.05.1990
Permanent home address	**:**	H.No. 230, Ward No. 8A Guru Teg Bahadur Nagar Dhuri

EDUCATIONAL QUALIFICATIONS

Bachelor degree	:	B.Sc. Agri. (Hons.)
University and year of award	:	Punjab Agricultural University, Ludhiana 2011
OCPA	:	6.73/10.00
Master's degree	:	M.Sc. Plant Breeding and Genetics
OCPA	:	6.67/10.00
Title of Master's Thesis	:	Irradiation induced mutagenesis in sugarcane