Genetic analysis of M_5 generation of Gamma irradiated red rice *(Oryza sativa L.)* Mutant lines

ABSTRACT

In rice, induced mutations play significant role in development of semi dwarf and high yielding ~~variants.~~ The genetic variability induced by four Gamma rays treatments was studied in traditional red rice variety *Kajejaya* in Randomized complete block design with two replications during *Kharif* 2021. Analysis of variance documented Gamma rays treatment brought significant variability and exhibited a wide range of values for ~~all traits~~ (Which traits like yield traits or biotic stress etc. Specify). Six superior mutants(T35-L-06, T35-L-17, T35-L-15, T45-L-07 T45-L-13 and T45-L-06) exhibited more number of productive tillers/plant, panicle length, number of filled grains/panicle, high test weight and higher grain yield/plant and T45-L-13, T45-L-06 and T35-L-15 mutants took lesser days to mature (114,117.5 and 118 days) and exhibited reduced plant height (84, 80 and 72cm) compared to untreated parent check. Induced mutations were effective in creating variability in plant height and maturity duration along with increased mean of reproductive components at higher doses (35kR and 45kR) as compared to lower doses (15kR and 25kR).

Keywords: Traditional red rice, Gamma rays, induced variability, Semi-dwarf, Earliness.

1. INTRODUCTION

Rice (*Oryza sativa* L.), belonging to genus *Oryza* of family *Poaceae* is also the other name for food in Asian countries and has become the Grain of Life. In India traditional red rice varieties are being grown since ancient times. People of Coastal Karnataka always have a special preference for red rice characterized by a red colour that adheres even after milling and the red bran layer is viewed as a rich source of minerals and vitamins. As compared to white rice red rice are higher iron and zinc content (Rathna Priya T. S. *et al.* 2019) and possess many special features like offering high degree of resistance to drought, salinity, submerge and also to storage insect pests (Chaudhary and Tran, 2001).

To cater the needs of ever increasing population, accomplishment of higher production target without compromising the nutritional quality for health concern, more variability is required with high yielding potentials that are also tolerant to biotic and abiotic stresses. Incidentally, market, demand for red rice is also expected to be increased due to their nutritional and nutraceutical properties. Therefore, accomplishment of vertical increment in production front is the only option left out for the day to offset the enormous challenges in meeting the demand for rice.

KAJEJAYA is a traditional rice variety having long bold red kernels is grown in all seasons in Coastal Karnataka and having special taste, medicinal value (promotes lactation, improve the digestion and repairs blood disorder and skin allergies) and nutritionally rich. K*ajejaya* matures in 115-120 days and due to susceptibility ~~to lodging yield is low.~~ (recheck) and give reference regarding the importance of kajejaya like total area grown, etc. merit and demerits of this variety and you need to do mutation breeding particular in this variety.

To address the above constraints, mutation are being used to upgrade the well adapted local varieties by changing one or two main characters that hinders their productivity and keeping their quality value with taste and texture of variety. Rice is diploid species, more chances of getting genetic variability which could be available for selection in M2 generation (Yusuff Oladosu *et al.*(2014). Feng Li *et al*. 2019, rated gamma rays is most commonly used radiations in rice. Gamma rays are known to influence plant growth and development by inducing cytological and morphogenetic changes in cells and tissues (María de la *et al*. 2022).

Give review reference/Earlier study (at least related to work e.g. Earliness and reduced ht.) showing following results due to mutation,

1. more number of productive tillers/plant,
2. panicle length,
3. number of filled grains/panicle,
4. high test weight and higher grain yield/plant
5. Earliness

6. dwarfism

Initially experiment was started with an aim to induce mutations to generate variation sources of this traditionally grown variety for yield improvement, reduce the plant height and earliness. Present experiment was carried out to elucidate information on genetic variability generated due to different doses of gamma irradiation (15, 25, 35, 45kR) in mutant lines of M_5 generation for grain yield and yield contributing traits.

2. MATERIAL AND METHODS

The experiment was conducted during *Kharif* 2021 at Zonal Agricultural and Horticultural Research Station, Brahmavar. Initially 200 seeds of long bold traditional red rice variety Kajejaya (popular in coastal Karnataka for nutrient values, cooking and eating characteristics) were exposed to lower (15kR and 25kR) and higher (35kR and 45kR) doses of gamma rays treatment from Cobalt 60 at BARC Mumbai. Plant to progeny method was followed to forward the individual plants from M_1 to M_2 and Semi-dwarf, earliness and medium bold red grain type that were primarily selected and forwarded to M_3 generation. Individual mutant plant progenies were selected from the M_4 generation based on normal and fertile plants from four different irradiation treatments during the previous (2020) year. The present study materials comprised 29, 22, 30 and 25 lines from the 15kR, 25kR, 35kR and 45kR gamma treatments respectively, and a total of 106 M_5 mutant lines with one untreated parent check (Kajejaya) were grown in *kharif* 2021. Randomized Complete Block Design (RCBD) with two replications was followed for conducting trial. The twenty-one days old healthy seedlings raised in wet nursery method were transplanted to the well-prepared puddled field from each treatment including the check (e.g. add check name). Normal spacing of 15 cm within a row and 20 cm between the rows was adopted in the field. Ten randomly selected plants in each mutant line were studied for recording observations.

Give details of Kajejaya e.g 1. Duration, plant type, grain type, etc. in tabular form.

The experimental data collected on 16 yield and yield attributing traits (Table 1) were subjected to standard statistical procedure as prescribed by Cochran and Cox (1957). The genotypic and phenotypic coefficients of correlation and path coefficients were analyzed by Windstar Version 9.2 from indostat services and Principal component analysis of 16 yield and yield attributing traits was done by R Studio, software 4.3.2.

3. RESULTS AND DISCUSSION

3.1 Analysis of variance and genetic variability

Results revealed that the mean sum of squares due to mutant lines were highly significant for all the traits studied, designating the gamma irradiation generated genetic variability in the experimental material. Wide range of values for all the traits like, plant height (53-97.50cm), panicle length (15-25cm), number of tillers per plant (11-31), number of productive tillers per plant (9-23), number of grains per panicle(70-150), number of filled grains per panicle(58-134), test weight (16.80-30.83g) and grain yield per plant (16.40-32.20g) in all mutant lines was evident (Table 1), suggesting a considerable induced variation in the quantitative characters in mutant lines of all treatments. Similar results were reported by K. Mahantashivayogayya *et al*. (2016).

Higher to moderate phenotypic and moderate genotypic coefficient of variations were observed on parameters covering number of tillers per plant (22.66 % and 18.86%), number of grains (19.65 %and 15.58%) and filled grains (21.84 %and 17.39%) per panicle, test weight (15.42 %and 12.30%), grain yield per plant (17.19 %and 13.22%) and kernel breadth (12.16 %and 11.45%), imply negligible influence of environment on these traits (Table 2). The findings were also on line with the observations of Pandey *et al.* (2018) and KP Deepthi *et al.* (2022). This indicates greater scope of selection for these traits. Therefore, presence of variations and the variability could be attributed solely by the gamma rays treatment and raises the hopes for further improvements as the effects of external environment was considerably low.

3.2 Heritability and Genetic advance

In the present study, high heritability was observed in time taken in terms of days for fifty per cent flowering (90.5%), days to maturity (86.5%), leaf length (94.4%), leaf width (95.5%), number of productive tillers per plant (70.37%) and kernel breadth (88.8%). This indicated that the induced variability in mutant population was fixed by selection due to an increased homozygosity of the genes involved (Khan and Qureshi, 2006).

Higher to moderate heritability coupled with moderate GAM (expand and make a list of abbreviations) was seen in plant height, panicle length, grain yield per plant and straw yield per plant, implying the least influence of environment. However, the facts of traits being governed by additive gene action inferred ample scope for genetic improvement in traits through selection. Here also it was evident that response to selection for yield attributing characters was directly proportional to the function of its genetic variance, heritability and genetic advance as described by Khan *et al.,* 2004. Further, the selection for quantitative traits, such as yield, should preferably be carried out in early generations (Sneepe,1977), as most of the desired combinations of favourable alleles were likely to be lost in advanced generations due to intensive or even no selection for other traits.

3.3 Character association

To group the genotypes into different clusters using 16 traits Heat map clustering was used (Fig. 1 and fig. 2). The cluster analysis grouped the genotypes into three main clusters (Brown, White and Green). The grain yield per plant exhibited positive and significant association with the traits covering days to fifty per cent flowering, number of tillers per plant, number of productive tillers per plant, number of grain per panicle, number of filled grains per panicle, spikelet fertility, test weight, straw yield per plant and kernel breadth at both phenotypic and genotypic levels. The results were also found in accordance with Fentie *et al.* (2021). Therefore, the selection of these traits would be beneficial in the process of yield improvement program.

Path analysis

To determine the direct and indirect effects of growth attributes (independent variables) on grain yield per plant (dependent variable) path analysis with pictorial presentation was performed (Fig, 3 and fig, 4). The results of path coefficient analysis revealed that nine traits showed direct positive effect on grain yield such as; days to fifty per cent flowering, leaf length, leaf width, panicle length, number of productive tillers per plant, number of grains per panicle, spikelet fertility, test weight and straw yield per plant. The highest direct positive effect was found for number of grains per panicle at both phenotypic and genotypic level, indicating the effectiveness of direct selection for these traits in improvement of grain yield per plant. These results were similar to the results depicted by Dhurai *et al.* (2016). In the present study, very low residual effect of 0.1422 indicated that the traits included in this study explained high percentage of variation in the grain yield due to gamma rays treatment**.**

3.4 Mean performance of five productive Mutant lines from each treatment

Five productive mutants from each treatment 15kR, 25kR, 35kR and 45kR ranked top, based on higher grain yield per plant as compared to check (24g). Table 2 showed that mutant plant that irradiated with higher dose of gamma rays radiation at 35kR and 45kR caused significant changes in the reproductive components such as number of productive tillers per plant, number of filled grains per panicle, panicle length, high test weight and higher grain yield per plant as compared to lower doses at 15kR and 25kR. Sellammal and Maheswaran (2013a) reported mutagenic efficiency and effectiveness was more at higher gamma rays doses.

The study revealed that T35-L-15, T45-L-13 and T45-L-06 lines took lesser days (114,117.5 and 118 days) for maturity with reduced plant height, (84, 80 and 72cm) more number of productive tillers per plant(19, 18 and 16), panicle length (23, 22 and 21cm), number of filled grains per panicle (129, 126 and 123), higher test weight (28.22, 27 and 26.12gm) and higher grain yield (28.8, 29.1 and 27.1gm) as compared to untreated parent check. The results might be due to the selection of normal looking plants in early generation leading to elimination of aberrant plants and also due to significant influence of gamma rays irradiation induced at the genetic level. Similar results were also noticed by Shehata *et al.,* 2009 for early maturity, Babaei *et al,* 2010 for thousand grain weights with effective tillers per plant and Singh, 2006 for grain yield per plant in rice. Three superior mutants like, T45-L-07, T35-L-06 and T35-L-17, showed reduced plant height (77.5, 76 and 78.5cm) more number of productive tillers per plant(19, 21.5 and 22.5), panicle length (24.5, 23 and 24cm), number of filled grains per panicle (133.5, 128 and 129), high test weight (30.28, 30.83 and 29.17gm) and higher grain yield (31.5, 32.2 and 30.9gm) as compared to untreated parent check. Observations suggested that irradiation with gamma rays could cause a genetic change in rice especially, for characters controlled by closely linked genes that could not be disassociated by gene recombination.

4. CONCLUSION

Gamma radiation could affect the morphological and agronomical characters of traditional local red rice variety. 35kR and 45kR gamma rays treatment induced more variability with respect to yield and yield contributing traits as compared lower dose (15kR and 25kR) of gamma rays. T35-L-15 and T45-L-13 mutant lines picked as trait of interest for early maturity and reduced plant height with increased mean value for reproductive components. These two mutant lines further tested in different environmental conditions for confirmation.

5. Future scope

List of abbreviations

Acknowledgments

Competing Interests

AUTHORS' CONTRIBUTIONS

Follow authors guidelines for reference writing.

1. In text it should be in number format
2. Check all reference pattern.

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Table 1. Analysis of Variance for yield and yield components in the mutant lines of M_5 generation of red rice variety Kajejaya

Source of Variation	Degrees of freedom	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}	X_{15}	X_{16}
Replication	1	4.20	1.68	0.67	0.01	65.06	0.46	0.56	1.86	113.7	42.17	8.91	1.01	6.74	3.71	0.006	0.0001
Mutant lines	106	21.79**	22.75**	21.9**	0.19**	77.04**	6.13**	27.75**	21.44**	814.69**	798.38**	15.07**	20.00**	26.49**	28.35**	0.006**	0.002**
Error	106	1.08	1.64	0.63	0.004	18.84	1.59	5.02	3.68	185.58	178.78	4.52	4.44	6.80	5.97	0.003	0.00017
CD (5%)		2.06	2.54	1.58	0.13	8.60	2.50	4.44	3.80	27.00	26.50	4.21	4.18	5.17	4.84	0.10	0.02
CD (1%)		2.72	3.36	2.09	0.17	11.38	3.31	5.88	5.03	35.73	35.07	5.57	5.53	6.84	6.41	0.14	0.03
CV (%)		1.39	1.05	3.26	4.72	6.69	6.60	12.55	14.10	11.97	13.21	2.40	9.29	10.98	7.23	6.88	4.07
Mean		74.86	122.06	24.41	1.40	64.83	19.15	17.86	13.59	113.77	101.18	88.48	22.67	23.72	33.79	0.79	0.32
Range	**Min**	71.50	114.00	18.00	0.85	53.50	15.00	11.00	9.00	70.00	58.50	75.82	16.80	16.40	26.45	0.60	0.27
	Max	87.00	129.50	32.50	1.95	97.50	25.00	31.00	23.50	150.00	134.00	92.53	30.83	32.20	42.60	0.90	0.40
PCV (%)		4.51	2.86	13.78	22.28	10.68	10.26	22.66	26.06	19.65	21.84	3.53	15.42	17.19	12.26	8.74	12.16
GCV (%)		4.29	2.66	13.38	21.77	8.32	7.86	18.86	21.91	15.58	17.39	2.59	12.30	13.22	9.89	5.39	11.45
H^2 broad sense (%)		90.5	86.5	94.4	95.5	60.7	58.7	69.3	70.7	62.9	63.4	53.8	63.6	59.1	65.2	38.0	88.8
GAM (%)		8.42	5.09	26.79	43.84	13.35	12.40	32.36	37.96	25.46	28.53	3.92	20.21	20.95	16.46	6.84	22.24

** Significance at 1% level, *significance at 5% level

Where,

X_1 - Days to fifty per cent flowering (days)
X_2 - Days to maturity (days)
X_3 - Leaf length (cm)
X_4 - Leaf width (cm)
X_5 – Plant height (cm)
X_6- Panicle length (cm)
X_7- Number of tillers per plant
X_8- Number of productive tillers per plant
X_9- Number of grains per panicle
X_{10}- Number of filled grains per panicle
X_{11}- Spikelet fertility (%)
X_{12}- Test weight (g)
X_{13}- Grain yield per plant (g)
X_{14}- Straw yield per plant (g)
X_{15}- Kernel length (cm)
X_{16}- Kernel breadth (cm)

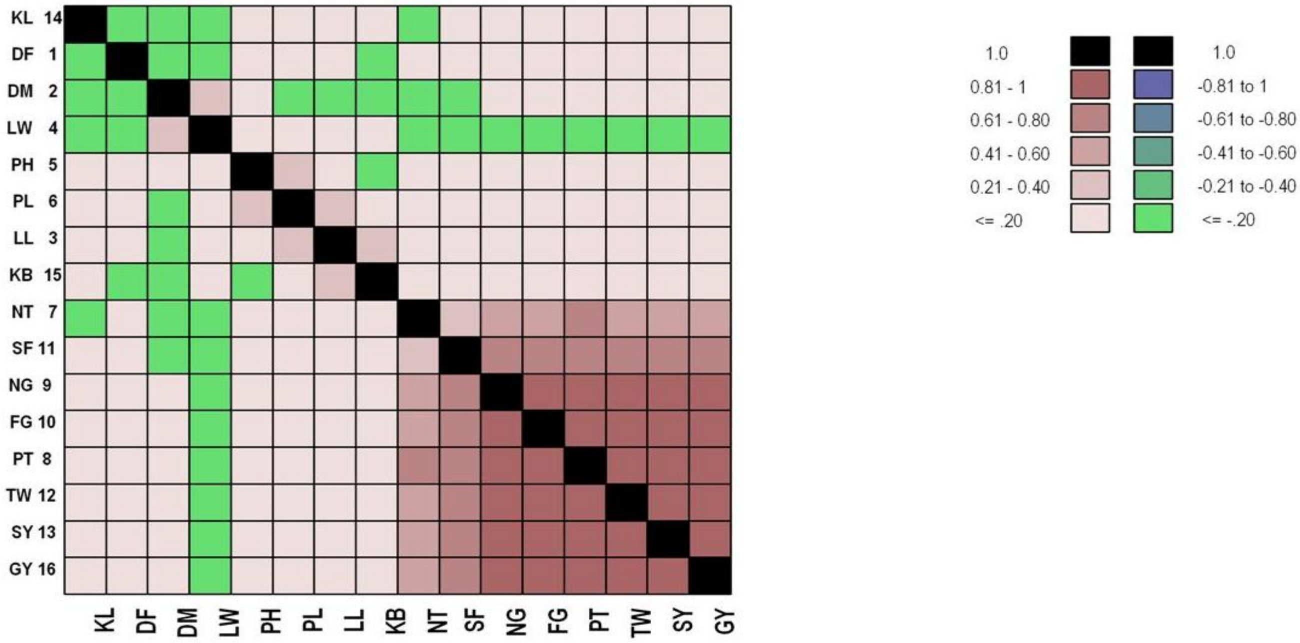

Fig1: Heatmap (phenotypic correlation) of 106 mutant lines and 16 traits in *kharif* season. Different colors (brown, white and green) and indicates the grouping of mutant lines into three main clusters.

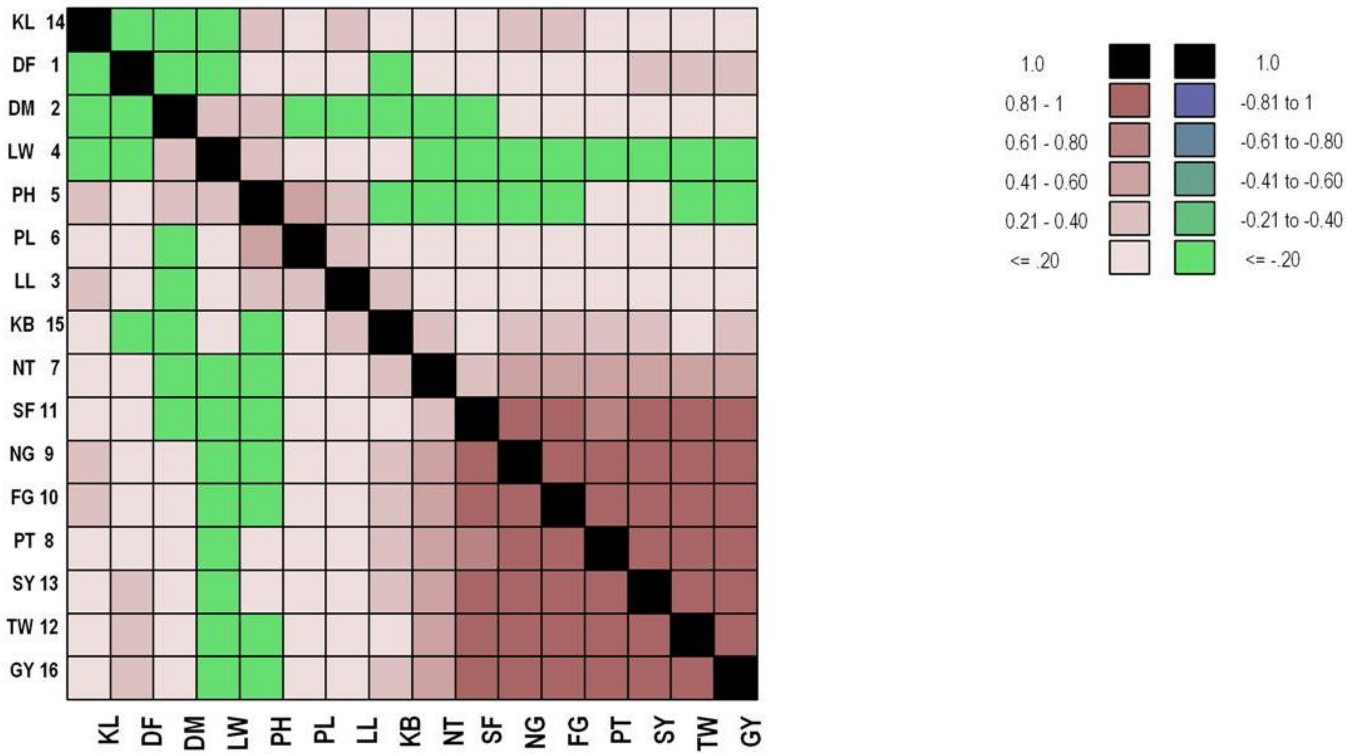

Fig 2: Heatmap (genotypic correlation) of 106 mutant lines and 16 traits in *kharif* season. DF- days to flowering, DM- days to maturity, LL- leaf length, LW- leaf width, PH- plant height, PL- panicle length, NT- number of tillers per plant, PT- number of productive tillers per plant, NG- number of grains per panicle, FG- number of filled grains per panicle, SF- spiklet fertility, TW- test weight, SY- straw yield per plant, KL- kernel length, KB- kernel breadth, GY- grain yield per plant. Different colors (brown, white and green) and indicates the grouping of mutant lines into three main clusters.

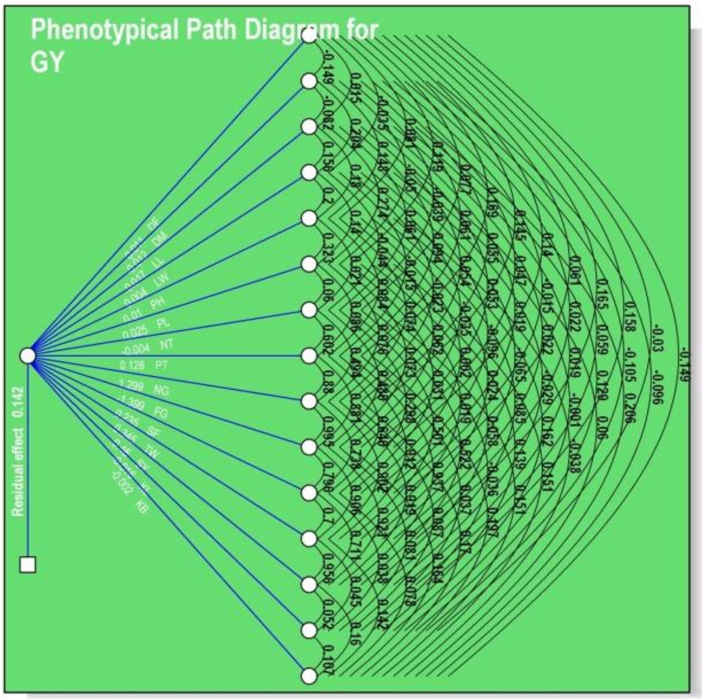

Fig 3: Phenotypic Path coefficient analysis determined the direct and indirect effects of studied traits on grain yield.

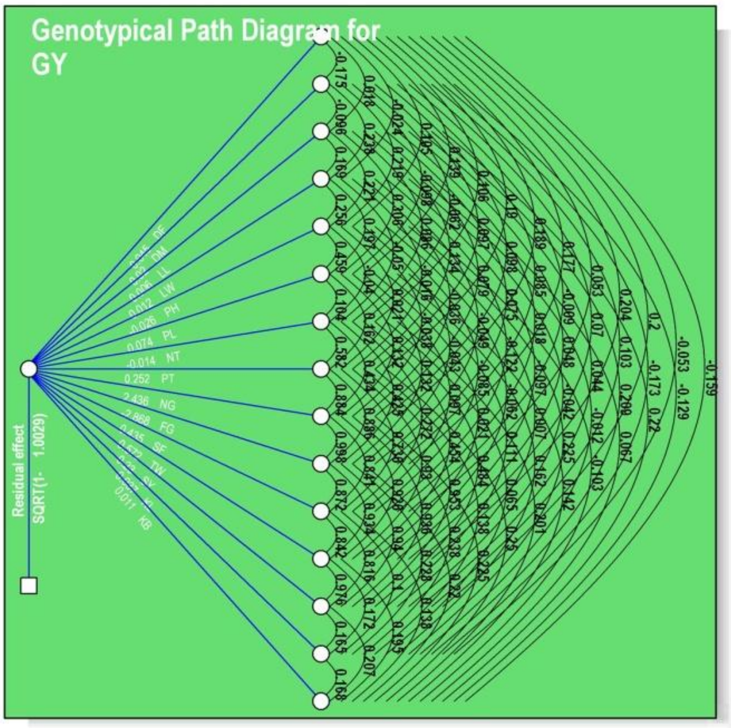

Fig 4: Genotypic Path coefficient analysis determined the direct and indirect effects of studied traits on grain yield. DF- days to flowering, DM- days to maturity, LL- leaf length, LW- leaf width, PH- plant height, PL- panicle length, NT- number of tillers per plant, PT- number of productive tillers per plant, NG- number of grains per panicle, FG- number of filled grains per panicle, SF- spiklet fertility, TW- test weight, SY- straw yield per plant, KL- kernel length, KB- kernel breadth, GY- grain yield per plant.

Table 2. Mean performance of top 5 productive mutant lines from each treatment based on higher grain yield in M_5 generation of red rice variety Kajejaya

Gamma rays treatment	Productive Mutant lines	Days to maturity	Plant height(cm)	Number of productive tillers per plant	Panicle length (cm)	Number of filled grains per panicle	Test weight (g)	Grain yield per plant (g)
15kR	T15-L-07	122.0	74.5	23.5	24.5	134.0	28.56	30.7
	T15-L-04	124.5	77.0	19.0	21.0	125.0	22.58	29.2
	T15-L-25	127.0	80.6	18.0	17.0	111.0	26.72	28.0
	T15-L-12	122.5	75.5	18.5	19.0	120.0	25.68	27.0
	T15-L-10	116.5	74.5	13.0	19.5	116.0	24.92	26.4
25kR	T25-L-12	123.5	79.0	21.0	17.5	116.0	27.84	29.3
	T25-L-17	128.5	73.5	18.0	22.0	125.5	26.95	28.1
	T25-L-16	126.0	81.5	17.0	21.5	123.5	26.75	27.7
	T25-L-14	122.5	80.0	16.0	20.5	122.5	26.26	26.8
	T25-L-04	123.5	86.0	14.5	18.0	111.0	23.57	24.9
35kR	T35-L-06	121.5	76.0	21.5	23.0	128.0	30.83	32.2
	T35-L-17	123.0	78.5	22.5	24.0	129.5	29.17	30.9
	T35-L-15	114.0	84.0	19.0	23.0	129.0	28.22	28.8
	T35-L-23	124.0	77.0	16.5	21.5	124.5	27.09	28.3
	T35-L-01	120.0	81.5	17.0	20.5	121.0	26.31	27.7
45kR	T45-L-07	120.5	77.5	19.0	24.5	133.5	30.28	31.5
	T45-L-20	123.5	71.5	19.0	24.0	130.0	28.41	29.6
	T45-L-13	117.5	80.0	18.0	22.5	126.0	27.50	29.1
	T45-L-06	118.0	72.0	16.0	21.0	123.0	26.12	27.1
	T45-L-23	122.0	82.0	14.0	22.5	123.5	23.61	24.6
Untreated parent check		120-125	90-95	12.0	18.5	106.0	24.25	24.0
Mean		122.06	64.82	13.59	19.15	101.18	22.67	23.72
CD (5%)		2.06	8.60	3.80	2.50	26.50	4.18	0.02
CD (1%)		2.72	11.38	5.03	3.31	35.07	5.53	0.03