Morphological and Physiological Traits of Maize in Response to Seed Treatment with gamma radiation

Abstract :

Maize holds considerable economic importance crop in Pakistan and mutation breeding can help develop improved varieties with desirable characteristics. Present study aimed to test influence of gamma rays on phenotypic, physiological and crop productivity. The research trial was conducted using RCBD with two replications. Seeds were exposed to four different gamma radiation intensities(20,40,60,80 Gy). The plants characters which were measured include : height of plant , leaf number, length of cob , kernals per cob , leaf surface area, leaf pigment concentration ,carotenoids as well as maturity characters. Statistical evaluation revealed that there were statistically meaningful variations among the treatments across all evaluated traits, which means that gamma irradiation was effective in inducing genetic variability in maize. In all the treatments, the T5 treatment proved better than other treatments in the majority of agronomic and yield related traits , whereas T4 treatment exhibited higher chlorophyll contents. Correlation and path coefficient assessment showed that height of plant, leaf surface area, length of tassel, content of pigments as well as carotenoids showed favorable association on grain yield. Results indicate that gamma irradiation has potential for maize breeding programmes for yield and physiological traits as a source of induced variability.

Keywords: Maize , gamma irradiation, mutation breeding, morphological traits, physiological traits, grain yield, genetic variability, chlorophyll content, crop improvement.

Introduction

The agriculture sector of Pakistan is very significant for providing food supply to the country , creating employment oppurtunities in rural areas, and stimulating industrial activities .Maize(Zea mays L) has recently gained importance in the agriculture sector of Pakistan due to its contribution towards human nutrition , animal and poultry feed, edible oil, starch , and other processed food supply ,and various agro-industrial chains.Pakistan's maize production has witnessed remarkable growth over the years, reaching 9.74 million metric tons in 2023 compared with only 1.66 million metric tons recorded in 2001..Punjab contributed around 77.45%of the total national maize output . However, there is still a need to improve productivity in order to supply high demand and decrease reliance on few high yielding areas within the country.

Maize is grown in various types of agro-climatic conditions in Pakistan. However, the highest production of maize occurs in Punjab and Khyber Pakhtunkhwa provinces. Maize is usually grown during spring and autumn seasons. The performance of maize is affected due to various factors like genotype, sowing time, temperature moisture, soil fertility and agronomic practices. Recent studies have shown water stress can cause significant impact on maize growth and production. Efficient nutrient and water management helps reduce production losses (Muhammad et al., 2022).Therefore, growth,adoption of high productivity as well as stress tolerant maize genotypes along the efficient agronomic practices are essential for sustainable maize production in Pakistan.

Despite having favorable agro-ecological conditions, maize productivity is highly susceptible to climatic change, water scarcity, heat stress, inefficient use of inputs and variation in access to improved seed and production technologies among farmers. Future climate change projections for Pakistan show increasing temperatures, with some climate change scenarios predicting decreased maize yields (Yasin et al., 2021). To increase resilience to climate change, adaptation strategies such as using heat and drought tolerant hybrids, changing planting dates, improving irrigation and fertigation techniques and improving soil and nutrient management systems is vital (Yasin et al 2021; Muhammad et al 2022).

Maize productivity and flexibility can be improved using traditional, molecular and mutation breeding techniques. Mutation breeding allows for novel genetic variation to be introduced

while maintaining much of the positive genetic material of an already adapted variety. This can allow breeders to change certain traits which may be difficult to alter by selection, such as agronomic, physiological and quality related traits. Physical mutagens like gamma irradiation and chemical mutagens such as Ethyl Methane Sulfonate (EMS) are often used to create genetic variability within crops. Mutant populations can then be investigated for any useful alterations to plant architecture, maturity, fertility, stress tolerance, disease resistance and yield related traits.

Gamma irradiation is capable of causing changes within the genome leading to visible cytological, morphological, physiological and agronomic differences. In recent times, research has shown in maize that mild and moderate levels of gamma irradiation can cause improvements in certain growth and yield traits within some genotypes. However, high doses can lead to decreased germination, plant vigour, plant height, fertility and yield related traits (Kikakedimau et al.,2022). Similar findings have been seen in rice where gamma irradiation have been used to cause mutations within rice which cause improved agronomic traits and also cause modifications in maintainer and restorer lines (Ghazy et al., 2023 ; Elsherbiny et al., 2024). This shows that the effectiveness of the irradiation will depend on the genotype, tissue/seed type, source of radiation, dose and trait.

Choosing the correct dose of mutagen is key to successful mutation breeding programmes. Using high doses can cause severe cellular damage leading to low germination, sterility and survival. Whereas if the dose is too low there may not be enough useful variation. Optimizing the dose should be done using germination %, seedling survival, reduction in growth, fertility and desired heritable phenotypes. Mutant populations should also be looked at over multiple generations to determine if any of the mutations caused are just physiological damage. Recent research has been done in creals to show how important it is to look at the early generations and see how the mutations have affected the plants, and then also look at the later generations to see how they have effected the agronomic traits (Kikakedimau et al., 2022; Ghazi et al.,2023;Elsherbiny et al., 2024).

Mutation breeding could be an effective method to compliment improving local maize genotypes in Pakistan. This can cause variation within the maize to cause early maturity, improved yield traits, imoroved water use efficiency, heat and drought tolerance, disease resistance and other traits. Using mutangenesis alongside field trails, quantitative genetics,

molecular analysis and modern selection methods can help speed up finding useful lines. Therefore looking at the effects of various mutagen treatments and doses on growth, development, fertility and productivity related characteristics in maize is important to create improved germplasm to help sustainable maize production in Pakistan.

MATERIALS AND METHODS

The field experiment was held in the research area of the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad . The materials used was Sultan variety of maize which was treated with gamma rays at four different doses i.e. 20, 40, 60 and 80 Gray.

Gamma irradiation was applied to dried seeds obtained from Nuclear Institute of Agriculture & Biology Faisalabad. Seeds were manually planted in the field. The field trial was established using RCBD with two replications. Two rows of 4 meter length were maintained for each treatment. Distances between rows and among plants within a row were maintained at 75 cm as well as 25 cm. Standard agricultural practices continued throughoutthe crop growth cycle.

Individual plant observations were made for the following agronomic and physiological traits: height of plant, time to tasseling, time to silking, length of tassel, leaf count, kernels per cob, length of cob, maturation period, Leaf surface area, and Physiological measurements. Chlorophyll as well as carotenoid measurements were carried out using the UV

spectrophotometer.



Statistical analysis

The statistical variance of each of these characters was calculated . The estimation of heritability was done through the calculation of Analysis of Variance and Genetic as well as phenotypic variance components. Path coefficient analysis was done to understand the effect of each of these characteristics on the yield either directly or indirectly by the use of genotypic correlations, with one of the characteristics as an assessment variable while others as causal variables.

The GCV and PCV were calculated using the following equations:

- $GCV = \left[\frac{\left(\sqrt{\delta^2_g}\right)}{X}\right] X\ 100$
- $PCV = \left[\frac{\left(\sqrt{\delta^2_p}\right)}{X}\right] X\ 100$

RESULTS AND DISCUSSION

The data of the following 13 characters of maize was analyzed. Different dose of gamma irradiation significantly affected nearly all the traits of maize plant (Viccini and Carvalho ,2002).

T	PH (cm)	DT	DS	TL (cm)	LPP	CPP	CL (cm)	GPC	DM	LA (cm)^2	Chl a	Chl b	Carotenoids
T1	128.33	29.81	**65.20**	**24.38**	**8.46**	**0.96**	**14.77**	**65.99**	**91.50**	**205.66**	**1.50**	**1.12**	**0.213**
T2	133.06	33.02	**64.12**	**29.81**	**8.71**	**1.12**	**15.45**	**80.83**	**92.90**	**264.31**	**0.76**	**0.65**	**0.160**
T3	133.68	33.96	**61.70**	**33.02**	**9.50**	**1.09**	**12.06**	**37.24**	**92.00**	**254.02**	**1.33**	**1.21**	**0.183**
T4	160.48	28.50	**59.42**	**28.50**	**8.26**	**1.04**	**13.26**	**84.81**	**94.68**	**319.31**	**2.16**	**1.68**	**0.196**
T5	194.35	24.38	**53.45**	**33.90**	**10.96**	**1.09**	**16.70**	**321.90**	**81.85**	**322.97**	**1.93**	**1.51**	**0.264**

Table 1: **Mean comparison table of all traits**

The mean comparison table showed considerable variation among all the gamma irradiation doses throughout the studied maize traits . All treatments had significant effects on growth, yield, maturity and physiological characters showing that gamma irradiation has been effective in creating variability between the plants. When compared to all the treatments, T5 was the superior treatment in the majority of important agronomic and yield-related traits. It showed maximum mean for plant stature, male inflorescence length, leaf count,ear length, kernels of cob, leaf area and carotenoids. The treatment of T5 also gave the highest yield of grain (321.90 grains/cob) indicating the most beneficial impact on productivity and plant growth. For flowering and maturity characteristics, the mean of T5 time to tasseling, time to silking and maturity duration were lowest, suggesting that it would flower and mature earlier than other treatments. Early maturity can be desirable as a shorter crop duration and potentially because plants may be able to avoid environmental stress conditions. Treatment T4 also exhibited better results in other physiological characteristics such as the highest contents of chlorophyll “a” and chlorophyll “b”. This means that under this illumination level photosynthesis activity is better and the physiological efficiency is improved. T4 also yielded in high leaf area with a moderate yield of grain. T1, however, tended to have lower mean values for many of the growth and productivity related characteristics such as height of plant, number of leaf, and kernels per cob.

Likewise, T3 had the lowest grain per cob and cob length, which was the poorest yield performance in this treatment. In general, the mean table revealed difference between the maize plants as a response to gamma ray exposure. For most of the traits, the superiority of the T5 treatment indicates that this treatment could be applied in breeding and mutation programs for improvement in the plant development, productivity and functional traits of maize.

Table 2 : Analysis of variance for evaluated characteristics

Traits	**Source of variation**	**DF**	**Sum of squares**	**Mean square**
Plant Height (PH)	Treatment	4	5913.16	1478.29*
	Replication	1	0.85	0.85
	Error	4	0.43	0.11
	Total	9	5914.43	
Days to Tasseling (DT)	Treatment	4	69.52	17.38*
	Replication	1	0.0828	0.0828
	Error	4	2.0788	0.5197
	Total	9	71.6843	
Days to Silking (DS)	Treatment	4	174.27	43.56**

	Replication	1	0.049	0.049
	Error	4	0.148	0.0371
	Total	9	174.471	
Tassel Length (TL)	Treatment	4	117.15	29.28*
	Replication	1	0.005	0.0053
	Error	4	1.162	0.2905
	Total	9	118.326	
Leaves Per Plant (LPP)	Treatment	4	9.725	2.431*
	Replication	1	0.00064	0.00064
	Error	4	0.02876	0.00719
	Total	9	9.7548	
Cobs Per Plant (CPP)	Treatment	4	0.03085	0.00771*
	Replication	1	0.00014	0.00014
	Error	4	0.00388	0.00097
	Total	9	0.03487	
Cob Length (CL)	Treatment	4	26.63	6.65*
	Replication	1	0.0009	0.0009
	Error	4	0.0739	0.0184
	Total	9	26.7048	
Grain Per Cob (GPC)	Treatment	4	106571	26642.7**
	Replication	1	0.00204	0.00204
	Error	4	0.17143	0.04286
	Total	9	106571	
Days to Maturity (DM)	Treatment	4	202.714	50.67*
	Replication	1	0.015	0.0154

	Error	4	0.553	0.1383
	Total	9	203.283	
Leaf Area (LA)	Treatment	4	19222.2	4805.54*
	Replication	1	0.50	0.50
	Error	4	2.2	0.54
	Total	9	19224.8	
ChL a	Treatment	4	2.40	0.60*
	Replication	1	0.095	0.095
	Error	4	0.030	0.0077
	Total	9	2.526	
ChL b	Treatment	4	0.012	0.003*
	Replication	1	1.967	1.967
	Error	4	1.38	3.47
	Total	9	0.01217	
Carotenoids	Treatment	4	0.012	0.003*
	Replication	1	1.967	1.967
	Error	4	1.38	3.47
	Total	9	0.01217	

ANOVA revealed that there were notable variations among gamma irradiation treatments for all the assessed maize characteristics, suggesting considerable genetic variability. The effect of different parameters was observed to be highly notable and notable , indicating that gamma irradiation significantly influenced vegetative growth, physiological as well as yield-related parameters. The mean plant stature varied between 128.33 and 194.35 cm with highly significant difference among the treatments. It is observed that the treatment of irradiation was beneficial to the vegetative growth as the highest height of plant was obtained in the treatment T5. The effect of irradiation on productivity was also apparent with grains per cob which showed high significance with all treatments, the T5 treatment having the highest grain yield (321.90 grains). Irradiation also affected time to silking with a range of 53.45 to 65.20 days. Time to tasseling and time to maturity were highly significant for treatments. T5 showed mostly early tasseling and maturity while flowering and maturity was delayed in other treatments. These results indicate the possibility of modifying flowering time and crop life cycle by irradiation. The length of the tassel also differed significantly with the maximum mean in T5 and T3, which suggested that it had the best reproductive growth in some irradiation doses. Number of leaf, number of cobs and cob length were found to be significantly affected by the treatments. Generally, T5 had the highest number of leaves/plant and longer cob.T5

generally had a higher number of leaf and longer cob. Cobs per plant and length of cobs are likely to be directly related to yield of grain. The differences in leaf area between the treatments were also large, T5 and T4 had the highest leaf area, suggesting that the irradiation treatments had an influence on the photosynthetic surface. The physiological response parameters exhibited significant differences. The highest chlorophyll contents were observed in T4 and T5 indicating enhanced photosynthetic ability and physiological performance under irradiated plants. T5 also had the highest carotenoid content, possibly suggesting greater tolerance to stress and pigment production when exposed to gamma irradiation. The overall mean and ANOVA results showed that gamma irradiation caused significant variation in the maize traits investigated. T5 was the best treatment with respect to most growth, physiological and yield traits namely height of plant, leaf surface area, length of cob, pigment contents as well as grains per cob. These traits may therefore be seen as selection criteria for future maize improvement and breeding programs.

Traits	M.S	G.M	GV	GCV %	PV	PCV %	EV	ECV %	h2bs %	GA %
ChB	0.31025	1.2386	0.102393333	28.7521485	0.105463333	29.17999472	0.00307	4.978558904	97.08904	44.675249
CL	6.65768	14.452	2.213086667	39.13228739	2.231506667	39.29480318	0.01842	3.570103744	99.17455	17.990718
CPP	0.00771	1.0627	0.002246667	4.597947209	0.003216667	5.501709929	0.00097	3.02120731	69.84456	6.5418722
Carte noids	0.00304	0.2035	0.001012176	7.052542946	0.001015649	7.064632007	3.47 E-06	0.413114259	99.65805	27.390304
ChA	0.60013	1.5427	0.197476667	35.77809016	0.205176667	36.4689488	0.0077	7.064877287	96.24714	49.596091
DM	50.6785	90.589	16.84673333	43.1241073	16.98503333	43.30075501	0.1383	3.90726941	99.18575	7.9192519
DS	43.5684	60.78	14.51043333	48.86071785	14.54753333	48.92314105	0.0371	2.470623721	99.74497	10.985052
DT	17.3807	53.799	5.620333333	32.32168067	6.140033333	33.78300386	5.20 E-01	9.828545597	91.53588	7.3991124
GPC	26642.7	118.15	8880.885713	866.9843891	8880.928573	866.9864811	0.04286	1.904623859	99.99952	139.98145
LA	4805.54	273.26	1601.666667	242.1018102	1602.206667	242.1426189	0.54	4.445379556	99.9663	25.698864
LPP	2.43135	9.18	0.808053333	29.66871061	0.815243333	29.80041331	0.00719	2.798614659	99.11805	17.109245
PH	1478.29	150.98	492.7266667	180.6522284	492.8366667	180.6723923	0.11	2.699209738	99.97768	25.799558
TL	29.2898	29.937	9.666433333	56.8236323	9.956933333	57.67115656	0.2905	9.850741661	97.08244	17.958593

Tale 4: genotypic associations among the studied maize characteristics

	DM	DS	DT	LA	CPP	LPP	PH	TL	ChA	CHB	Car	Grain yield
CL	-0.69**	-0.36*	-0.63**	0.20	0.15	0.45*	0.55**	0.05	-0.04	-0.16	0.54**	0.76**
DM		0.76**	0.82**	-0.36*	-0.24	-0.93**	-0.76**	-0.55**	-0.27	-0.26	-0.86**	-0.93**
DS			0.93**	-0.85**	-0.37*	-0.79**	-0.96**	-0.69**	-0.66**	-0.69**	-0.76**	-0.87**
DT				-0.77**	-0.15	-0.69**	-0.99**	-0.41*	-0.69**	-0.64**	-0.87**	-0.97**
LA					0.54**	0.43*	0.86**	0.60**	0.58**	0.62**	0.36*	0.62**
CPP						0.49**	0.35*	0.88**	-0.38*	-0.32	-0.22	0.29
LPP							0.71**	0.81**	0.20	0.25	0.71**	0.83**
PH								0.56**	0.63**	0.62**	0.76**	0.93**
TL									0.05	0.18	0.24	0.52**
ChA										0.99**	0.67**	0.43*
ChB											0.62**	0.39*
Car												0.84**

Abbrevations

CL= cob length, DM= days to maturity, DS= days to silking, DT= days to tasseling, LA= leaf area, CPP(# of cobs per plant), LPP(# of leaves per plant), Ph= plant height, TL= tassel length, ch a= chlorophyll "a", Ch b= chlorophyll"b", Car= carotene, *significant at 0.05, **highly significant at 0.01

Table 5: Phenotypic association among different charateristics of maize

	DM	DS	DT	LA	CPP	LPP	PH	TL	ChA	CHB	Car	Grain yield
CL	-0.67**	-0.36	-0.62**	0.20	0.12	0.45**	0.55*	0.05	-0.04	-0.16	0.54**	0.76**
DM		0.76**	0.78**	-0.36	-0.22	-0.93**	-0.76**	-0.54**	-0.27	-0.26	-0.86**	-0.93**
DS			0.91**	-0.84**	-0.34	-0.79**	-0.96**	-0.69**	-0.65**	-0.69**	-0.76**	-0.87**
DT				-0.75**	-0.14	-0.67**	-0.97**	-0.41*	-0.65**	-0.61**	-0.84**	-0.93**
LA					0.47**	0.43*	0.86**	0.60**	0.57**	0.61**	0.36	0.61**
CPP						0.44*	0.30	0.77**	-0.36	-0.21	-0.19	0.26
LPP							0.71**	0.79**	0.18	0.25	0.70**	0.83**
PH								0.56**	0.62**	0.61**	0.76**	0.92**
TL									0.036	0.18	0.23	0.51**
ChA										0.97**	0.66**	0.42*
ChB											0.61**	0.38
Car												0.85**

Abbrevations

CL= cob length, DM= days to maturity, DS= days to silking, DT= days to tasseling, LA= leaf area, CPP(# of cobs per plant), LPP(# of leaves per plant), Ph= plant height, TL= tassel length, ch a= chlorophyll "a", Ch b= chlorophyll"b", Car= carotenoids , **highly significant at 0.01, *significant at 0.05

PATH COEFFICIENT ASSESSMENT

Path coefficients represent standardized regression estimates. Therefore , this technique can be used to identify which traits would be the most useful to select for when improving plant performance . Path coefficient assessment is a statistical techniquethat can be used to identify the contribution of different traits on grain productivity in maize. Characters were found that had favorable and direct effect on grain productivity meaning that they are important traits to select for. There was a large desirable effect on grain productivity(0.49184). Height of plant influenced notable indirect contribution on grain productivity. Cob number,pigment concentration and duration of maturity also showed positive indirect effect. Taller plants showed desirable contribution on improving grain yield. Leaf count led to notable direct contribution(0.2397) on grain yield. It resulted positive indirect contribution on maturity duration, chlorophyll contents and carotenoids. Cob length revealed favorable direct contribution on grain productivity(0.17421). Longer cobs lead to higher yields. Leaf surface area also had a positive effect (0.15268). More leaf surface area will result in more grain yield. Male inflorescence length showed notable direct contribution(0.29381) on productivity. Physiological traits all had desirable direct contribution on grain productivity. Chlorophyll and carotenoid contents had favorable direct effect.Carotenoids had a relatively large notable direct effect (+0.10374). Chlorophyll a as well as chlorophyll b showed multiple favorable indirect effects on improving yield. This shows photosynthetic pigmets are important for improving yield in maize. Time to silking (-0.65287) and time to maturity (-0.47581) had a negative effect on grain yield. This shows that increasing flowering and maturity will decrease grain yield. Most characters influenced negative indirect effect. Days to tasseling had very small positive direct effects so there was a negative association with grain productivity. Overall plant stature,leaf count, male inflorescence length, ear length, leaf surface area, chlorophyll contents as well as carotenoids were some of the most important traits to improve grain yield. These characters could be useful to select for in the future when improving maize.

Table 6: Direct and indirect effect of all traits on all characters studies on grain yield

Traits	**Dirrect Effect On Grain yeild**	**Major Positive Indirect effects**	**Major Negative Indirect effects**	**Overall Effects On grain yield**
Plant Height(PH)	0.49184	LA , CPP , Chl “b” , DM	LPP, Carotenoids	Strong positive
Days to Tasseling(DT)	0.00752	TL ,LA,PH	DM, LPP, ChLs,carotenoids	Negative association
Days to Silking(DS)	0.65287	DM, CL , PH	LPP, LA,ChL “a”	Strong negative
Tassel Length(TL)	0.29381	TL ,ChL “b”, LPP	ChL “a”,DS	Positive
Leaves Per Plant (LPP)	0.23970	DM,PH,ChL “b”, carotenoids	LA	Positive
Cobs Per Plant(CPP)	0.13281	ChL “b”,TL,LA	DS	Positive
Cob Length(CL)	0.17421	CPP,ChL “b”, ChL “a”, DM	LPP,DS	Positive
Days to Maturity(DM)	0.47581	DT	LA,TL, Carotenoids	Positive
Leaf Area (LA)	0.15268	DS,LPP,CPP	PH,TL	Negative
Chlorophyll “a”(ChA)	0.05461	DM,LPP,CPP	DS	Positive
Chlorophyll “b” (ChB)	0.03249	DS,LPP,CL	TL, Carotenoids	Positive
Carotenoids	0.10374	LPP	PH,LA	Positive

Conclusion :

This study has demonstrated that gamma-irradiation possessed a notable growth responses ,physiological, maturity and productivity traits. Analysis of variance confirmed variability between treatments for all characters studied. This shows that gamma irradiation can be an effective way to produce useful genetic variation. The mean results showed T5 to be the most effective treatment for many of the most important agronomic traits. These included plant stature, male inflorescence, leaf count, ear length,kernels of cob, leaf area and carotenoid content. In addition,the T5 variety also tasseled,silked and matured early compared to the other varieties, meaning it is superior in terms of yield and earliness. The evidence for chlorophyll pigments revealed an improvement in physiological efficiency for T4. Correlation and path coeffiecient assessment showed that plant stature , leaf number, leaf surface area, tassel length, cob length, chlorophyll pigments and carotenoids were favorable contributor for grain productivity. However, phenological parameters had negative contributors for negative contributors for productivity. Plant stature ,leaf area , ear length ,leaf number, chlorophyll contents as well as carotenoids are all potential traits to assess how effective Improvements to grain yield have been within future maize breeding and mutation programs.

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