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Mutagenic effects of gamma rays on morpho-physiological traits of maize (*Zea mays* L.)

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Abstract

In Pakistan after wheat and rice maize is the third most important cereal. It is staple food in many countries of the world. . It is grown from latitude 58° north to 40° South with annual rain fall ranging from 250 to 5000 mm. The temperature between 25 to 28°C is optimum for germination of maize. About 70% of total production of maize is used as fodder and animal feed while 30% is used by humans in different forms. The experiment material comprised of maize variety Sultan treated with four gamma irradiation doses which are 20, 40, 60 and 80 Gray. Dry seeds were treated with above mentioned doses of gamma rays at Nuclear Institute of Agriculture and Biology Faisalabad, using Cobalt-60 as a source. Seeds were dibbled in the field. Experiment was laid out in randomized complete block design, with two replications. Observations on all plants were recorded on individual plant basis for agronomic and physiological characteristics i.e. plant height, days to tasselling, days to silking, tassel length, number of leaves per plant, number of cobs per plant, cob length, number of grains per cob, days to maturity, leaf area, chlorophyll "a", chlorophyll "b" and carotenoid. The results showed that morphological and physiological traits of maize could be improved by using gamma radiation. Gamma radiations were found to be good source for creating variation which are helpful in crop improvement programme.

Keywords: maize, mutation, variability, gamma radiation, yield and yield components
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Introduction

Pakistan is an agricultural country and people of the country directly and indirectly involved with agriculture sector. Most of the industry is agro-based. Agriculture has always been first priority for administration to boost up the production of cereals, fodder and cash crops. Cereals play crucial role in Pakistan agriculture. Maize is an important cereal crop. It is grown from latitude 58° north to 40° South with annual rain fall ranging from 250 to 5000 mm (Downsell *et al*., 1996). Maize is widely grown crop in the world with almost 50% production from USA. China, France and Brazil are next top producing countries. About 70% of total production of maize is used as fodder and animal feed while 30% is used by humans in different forms. It has been estimated that demand of maize will greatly increase in 2020 as compared to wheat and rice (Sohail *et al*., 2006).

In Pakistan after wheat and rice maize is the third most important cereal. It is staple food in many countries of the world. It was grown on an area of 0.94 million hectares with annual production of 3.34 million tons and average grain yield of 3.56 ton ha^{-1} in Pakistan (Pakistan economic survey, 2012-2013). Its significance is obvious because of its use as edible oil and high valued food for human beings, feed for livestock and poultry and a raw material for diverse agro-based industries (Chaudhry, 1983). Although the soil and climatic conditions of Pakistan are favourable to the production of maize, but the average yield of maize is lower than the other major maize producing countries of the world. Many factors such as varietal inherent potential, optimum plant population, irrigation, nutrition and pest management are responsible for the low production of maize. In Pakistan maize can be successfully cultivated twice a year in autumn as well as spring seasons. The temperature between 25 to 28°C is optimum for germination of maize (Basra *et al*., 2011).

Mutation breeding is important for bringing variation in one or two characters without modifying whole genetic profile of organism. Mutation has lot of potential to bring genetic changes in major crops like wheat, rice and maize (Mahandjiev *et al*., 2001). Higher gamma irradiation dose is directly proportional to number of branches per plant and dry weight which causes significant increase in branches and weight of vegetative parts after sowing of wet seeds. Mutation breeding is emerging as effective tool for crop improvement in relation to cytological changes (Acharya *et al*., 2007). Gamma rays produce cytological and morphological changes in tissues and change growth and development pattern (Gunckel and Sparrow, 1961). Gamma rays and other mutagens can be beneficially applied to develop new varieties in short duration while other conventional breeding methods take long period in variety development process (Khatri *et al*., 2005). Effectiveness of mutagenic treatments in crop improvement depends on mutagenic efficiency (mutation which produces desirable changes) so selection of effective mutagens and their doses are necessary for creation of desirable changes (Solanki and Sharma, 1994). Two mutagens acting in sequence one after the other produces different results as compared to the two mutagens which compete for their action on same site (Astveit *et al*., 1967). Such observations are useful in determining dose strength combination to get beneficial results. Mutation is heritable change and it can be produced naturally or induced by artificial mutagens in different seed or vegetative propagation. For study of structural and functional changes in plants gene induced mutation is becoming popular in field of cytology, molecular biology and biotechnology. Induced mutation produced genetic changes in cereals and other crops and is effective in the development of various cultivars of cereals and other crops (Lee *et al*., 2002).

Mutation has become an important tool in the development of varieties in short duration by generating variation in existing cultivars. Almost 64% of mutant varieties are developed by gamma irradiation (Ahloowalia *et al*., 2004). Mutation breeding is emerging as valuable tool in crop improvement in recent years. Mutagens play crucial role in breaking of linkage and hence play important role in crop improvement (Shah *et al*., 2008). Different polygenic traits on which crop improvement depends can be evaluated by using induced mutation. Developing embryo is suitable in study to evaluate the comparative analysis of change in genetic effects which are caused by the gamma rays and ethyl methane sulfonate. Comparison of these effects may helpful in determining linkage between endosperm and other seedling markers to point out exact genetic changes (Chatterjee *et al.,* 1965).

Materials and methods

Present studies were carried out in research area of the department of Plant Breeding and Genetics, University of agriculture, Faisalabad, during summer 2013. The experiment material comprised of maize variety Sultan treated with four gamma irradiation doses which are 20, 40, 60 and 80 Gray. Dry seeds were treated with above mentioned doses of gamma rays at Nuclear Institute for Agriculture and Biology (NIAB) Faisalabad, using Cobalt -60 as a source. Seeds were dibbled in the field. Experiment was laid out in randomized complete block design, with two replications. Each treatment comprises two lines, 4 m in length. Row to row and plant to plant distances were 75cm and 25 cm respectively. Recommended cultural and agronomic practices were applied throughout the growing period of the crop. Observations on all plants were recorded on individual plant basis for following agronomic and physiological characteristics. Chlorophyll and carotenoid contents were calculated by using UV-spectrophotometer. Plant height (PH; cm), Days to tasselling (DT), Days to silking (DS), Tassel length (LT; cm), Number of leaves per plant (LPP), Number of cobs per plant (CPP), Cob length (CL), Number of seeds per cob (GPC), Days to complete maturity (DM), Leaf area (LA; cm^2), Chlorophyll a (CL “a”), Chlorophyll b (CL ”b”) and Carotenoids (CR) contents were estimated in this study.

Statistical analysis

The data of each character was statistically analyzed for analysis of variance (Steel *et al.* 1997). Heritability estimates was calculated by analysis of variance. Genotypic and phenotypic components of variance were measured according to Cochran and Cox (1957). Path coefficient analysis was performed (Dewy and Lu, 1959) to assess the direct and indirect effect on yield using genotypic correlation where association of all the above traits were calculated by keeping one at a time as response variable and other contributing traits as casual variables.

Results and discussion

Analysis of variance (Table 1) revealed that highly significant differences for PH, DT, DS, LT, LPP, CPP, CL, GPC, DM, LA, CL “a”, CL “b” and CR were found between treatments of different gamma irradiations. The treatments showed sufficient range of variability PH, DT, DS, LT, LPP, CPP, CL, GPC, DM, LA, CL “a”, CL “b” and CR. Mean value for PH ranged from 128.33 to 194.35 days. Maximum mean value was observed for non-irradiated plants followed by 80 Gy gamma irradiated plants. Mean value for DT ranged from 56.2 to 49 days. Maximum mean value was observed for 60 Gy gamma irradiated plants followed by 40 Gy gamma irradiated plants. Range for DS was from 53.4 to 65.2 days. Mean value of plant treated with 20 Gy gamma irradiations showed maximum mean value after that plants treated with 40 Gy gamma irradiations showed highest value. Wali *et al.* (2006) also found significant differences among genotypes for DS. Mean value for GPC ranged from 37.24 to 321.90 grains. Maximum mean value was observed for non-irradiated plants followed by 80 Gy gamma irradiated plants. Viccini and Carvalho (2002) concluded that different dosage of gamma irradiation had significant effects on almost all traits of maize plant. Ali *et al,* (2014); Pavan *et al*. (2011) reported that different gamma irradiations affected different parameters i.e. LT, LPP, CPP, CL, DM, LA, CL “a”, CL “b” and CR.

Results shows that coefficient of variability of treatments for PH, DT, DS and GPC was 0.22%, 1.34%, 0.32% and 0.18% respectively. From Table 2 it was found that phenotypic coefficient of variation (180.67%) for PH was higher than genotypic (180.65%) and environmental (2.69 %) coefficients of variation. Phenotypic coefficient of variation (33.78 %) for DT was higher than genotypic (32.32 %) and environmental (9.82 %) coefficients of variation. Broad sense heritability for PH, DT, DS, and GPC was estimated highly significant (99.9 %) with genetic advance (17.95 %), highly significant (9.53 %) with genetic advance (7.39 %), highly significant (99.74 %) with genetic advance (10.98 %), and highly significant (97.08 %) with genetic advance (17.95 %) respectively. Higher broad sense heritability indicated that different irradiation doses affected plants for PH, DT, DS and GPC. Similarly results of LT, LPP, CPP, CL, DM, LA, CL "a", CL "b" and CR for CV, GA, GCV, PCV and h^2bs are indicated in Table 2.

Mean comparison test for PH following LSD showed that means of all treatments were significantly different from each other. It was recommended that selection of different mutants might be useful for the development of maize hybrids and synthetic varieties with improved plant height. Results showed that PH decreased by increasing dose rate. The results were similar to the findings of Irfaq and Nawab (2001). Khan *et al.* (2005) found that gamma irradiation affected the PH significantly. Malik *et al*. (2007) found that there were significant differences among genotypes for PH. Mean comparison test for DT, DS, LT, LPP, CPP, CL, GPC, DM, LA, CL "a", CL "b" and CR showed that means of non-treated and gamma irradiated plants were significantly different at different level of doses. Jayakumar *et al. (*2007) also found significant differences among various varieties of maize for DT. Ali *et al* (2014) also found the similar results that LPP decreased by increasing dose rate. Ei-Shouny *et al.* (2005) also found significant differences among genotypes for CL. Stoeva *et al.* (2007) found that gamma irradiation had significant effects on GPC. Shah *et al.* (2006) found that mutagens influenced the chlorophyll contents significantly. Navin and Agarwal (1997) reported that CL "a" and CL "b" were significantly affected by UV radiations. These results are shown in Table 3.

Estimation of genotypic and phenotypic correlations provide a measure of the extent of relationship occurring between two or more independent variables. Correlation analysis in plant breeding reveals the relative importance of different plant traits, which can be of value in a crop breeding programme. Genotypic and phenotypic correlation coefficient were worked out among all possible combinations of various characters. The Table 4 indicated the results that there is highly significant genotypic (0.92504) and phenotypic correlation (0.9249) between PH and GPC. PH highly significant correlated with LT, CL "a", CL "b" and CR at both genotypic and phenotypic level. The results are in agreement with Zarei *et al.* (2012) and Sadek *et al.* (2006). Chaudhary (1989); Krishan and Natarajan (1995); Annapurna *et al.* (1998) found that there was positive significant correlation between PH and GPC. DT had negative and significant correlation with GPC, LA, LPP, CPP , PH, LT, CL "a", CL "b" and CR at both genotypic and phenotypic. Tahir *et al.* (1991) concluded that number of DT were positively correlated with grain yield. Netaji *et al*. (2000) found that association of DT with GPC was non-significant. Jayakumar *et al.* (2007) explained that DT had high positive effects on grain yield. DS had negative and significant correlation with GPC and CPP at both genotypic and phenotypic levels. DT showed positive and highly significant correlation with DS at both genotypic and phenotypic levels while LA, LPP, PH, LT, CL "a", CL "b" and CR showed negative and highly significant correlation with DM at both genotypic and phenotypic levels. Debannath and Khan (1991) concluded that DS was positively correlated with GPC in maize. Ei-Shouny *et al.* (2005) explained that GPC was positively correlated with DS and this association was significant. Wali *et al.* (2006) found that GPC was negatively correlated with DS at both genotypic and phenotypic levels.

Correlation between LT, LPP, CPP, CL, GPC, DM, LA, CL "a", CL "b", CR and other traits results are shown in Table 4.

The path coefficient analysis technique was used by Dewey and Lu (1959). It is basically a consistent fractional regression coefficient and as such calculates the effects of every variable on the resultant variable directly as well as indirectly by partitioning the genetic correlation coefficient. It helps in selection of plant performance parameters. In this study direct and indirect effects of 13 traits were analyzed keeping grain yield as dependant variable. A positive and direct effect (0.49184) was observed of PH on grain yield (Table 5). DT, DS, LT, LPP, CPP, CL, DM, LA, CL "a" and CL "b" exhibited the positive indirect effect while LPP and CR exhibited negative indirect effect through PH on grain yield. PH had the maximum positive indirect effect (0.46823) via LA. The results are similar with the findings of Rahman *et al*. (1995), Singh *et al.* (1999), Umakanth and Khan (2001) and Malik *et al.* (2007). Gautam *et al*. (1999) concluded that PH had direct positive effects on grain yield. Similar findings were shown by Singh *et al.* (1999) which demonstrated the direct positive effects of PH and cob diameter on grain yield. Samonte *et al.* (2005) found the direct positive effects of PH on grain yield. The data in Table 5 revealed that a positive direct effect of DT on grain yield. There was a positive indirect effect via LA, PH and LT while a negative indirect effect of CL, DS, DM, CPP, LPP, CL "a", CL "b" and CR was observed on GPC. The present results are similar with the results of Martin and Russel *et al.* (1984). Negative direct effect of DS on grain yield was observed. DM, PH, CL, CPP and chlorophyll showed positive indirect effects on grain yield through DS while DT, LA, LPP, LT,CL "a" and CR showed negative indirect effects on grain yield. Singh *et al.* (2003) reported that DS had positive direct effect on grain yield, these results contradicts with our findings. These may be due to different genotypes or due to different environment. Different researchers are found different results for these parameter direct and indirect effect on grain yield. Similarly the results are shown in Table 5 for direct and indirect effects of LT, LPP, CPP, CL, DM, CL ”a”, CL “b” and CR on grain yield.

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Table 1: Analyses of variance for different yield components of maize

SOV	DF	PH	DT	DS	TL	LPP	CPP	CL	GPC	DM	LA	CL "a"	CL "b"	CR
Treatment	4	1478.29**	17.38*	43.56**	29.28*	2.43135*	0.00771*	6.65*	26642.7**	50.67*	4805.54*	0.60*	0.31*	0.003*
Replication	1	0.85	0.0828	0.0490	0.0053	0.00064	0.00014	0.0009	0.00204	0.0154	0.50	0.095	0.02015	1.967
Error	4	0.11	0.5197	0.0371	0.2905	0.00719	0.00097	0.0184	004286	0.1383	0.54	0.0077	0.003	3.47

Note: Plant height (PH), Days to tasselling (DT), Days to silking (DS), Tassel length (LT), Leaves per plant (LPP), Cobs per plant (CPP), Cob length (CL), Grains per cob (GPC), Days to complete maturity (DM), Leaf area (LA), Chlorophyll a (CL "a"), Chlorophyll b (CL "b") and Carotenoids (CR)

Table 2: Variability estimates for different yield components of maize

	PH	DT	DS	TL	LPP	CPP	CL	GPC	DM	LA	CL "a"	CL "b"	CR
CV (%)	0.22	1.34	0.32	1.80	0.92	2.93	0.94	0.18	0.41	0.27	5.69	4.47	0.92
GA (%)	25.79	7.39	10.98	17.95	17.10	6.54	17.99	139.98	42.66	25.69	49.59	44.67	27.39
GCV (%)	180.65	32.32	48.86	56.82	29.66	4.59	39.13	866.98	43.12	242.10	35.77	28.75	7.05
PCV (%)	180.67	33.78	48.92	57.67	29.80	5.50	39.29	866.98	43.30	242.14	36.46	29.17	7.06
h^2bs (%)	99.97	91.53	99.74	97.08	99.11	69.84	99.17	99.9	99.18	99.96	96.24	97.08	99.65

Note: Coefficient of variance (CV), Genetic advance (GA), Genotypic Coefficient of Variance (GCV), Phenotypic Coefficient of Variance (PCV), Broad Sense Heritability (h^2bs) Plant height (PH), Days to tasselling (DT), Days to silking (DS), Tassel length (LT), Leaves per plant (LPP), Cobs per plant (CPP), Cob length (CL), Grains per cob (GPC), Days to complete maturity (DM), Leaf area (LA), Chlorophyll a (CL "a"), Chlorophyll b (CL "b") and Carotenoids (CR)

Table 3: Mean comparison of different mutagenic treatments

S. No.	Treatment	PH	DT	DS	LT	LPP	CPP	CL	GPC	DM	LA	CL "a"	CL "b"	CR
1	T1	128.33(E)	29.81(A)	65.20(A)	24.38(C)	8.46(D)	0.96(B)	14.77(C)	65.99(D)	91.50(C)	205.66(E)	1.50(B)	1.12(C)	0.21(B)
2	T2	133.06(D)	33.02(A)	64.12(B)	29.81(B)	8.71(C)	1.12(A)	15.45(B)	80.83(C)	92.90(B)	264.31(C)	0.76(C)	0.65(D)	0.16(E)
3	T3	138.68(C)	33.96(A)	61.70(C)	33.02(A)	9.50(B)	1.09(A)	12.06(E)	37.24(E)	92.00(BC)	254.02(D)	1.33(B)	1.21(C)	0.18(D)
4	T4	160.48(B)	28.51(B)	59.42(D)	28.50(B)	8.26(D)	1.04(AB)	13.26(D)	84.81(B)	94.68(A)	319.31(B)	2.16(A)	1.68(A)	0.19(C)
5	T5	194.35(A)	24.38(C)	53.45(E)	33.96(A)	10.96(A)	1.09(A)	16.71(A)	321.9(A)	81.85(D)	322.97(A)	1.93(A)	1.51(B)	0.26(A)

Note: Plant height (PH), Days to tasselling (DT), Days to silking (DS), Tassel length (LT), Leaves per plant (LPP), Cobs per plant (CPP), Cob length (CL), Grains per cob (GPC), Days to complete maturity (DM), Leaf area (LA), Chlorophyll a (CL "a"), Chlorophyll b (CL "b") and Carotenoids (CR)

Table 4: Genotypic and Phenotypic correlation among different traits irradiated at different levels

		DM	**DS**	**DT**	**LA**	**CPP**	**LPP**	**PH**	**LT**	**CL "a"**	**CL "b"**	**CR**	**GPC**
CL	G*r*	-0.69**	-0.36*	-0.63**	0.20	0.15	0.45*	0.55**	0.05	-0.04	-0.16	0.54**	0.76**
	P*r*	-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	G*r*		0.76**	0.82**	-0.36*	-0.24	-0.93**	-0.76**	-0.55**	-0.27	-0.26	-0.86**	-0.93**
	P*r*		0.76**	0.78**	-0.34*	-0.22	-0.93**	-0.76**	-0.54**	-0.27	-0.26	-0.86**	-0.93**
DS	G*r*			0.93**	-0.85**	-0.37*	-0.79**	-0.96**	-0.69**	-0.66**	-0.69**	-0.76**	-0.87**
	P*r*			0.91**	-0.84**	-0.34	-0.79**	-0.96**	-0.69**	-0.65**	-0.69**	-0.76**	-0.87**
DT	G*r*				-0.77**	-0.15	-0.69**	-0.99**	-0.41*	-0.69**	-0.64**	-0.87**	-0.97**
	P*r*				-0.75**	-0.14	-0.67**	-0.97**	-0.41*	-0.65**	-0.61**	-0.84**	-0.93**
LA	G*r*					0.54**	0.43*	0.86**	0.60**	0.58**	0.62**	0.36*	0.62**
	P*r*					0.47**	0.43*	0.86**	0.60**	0.57**	0.61**	0.36	0.61**
CPP	G*r*						0.49**	0.35*	0.88**	-0.38*	-0.32	-0.22	0.29
	P*r*						0.44*	0.30	0.77**	-0.36	-0.21	-0.19	0.26
LPP	G*r*							0.71**	0.81**	0.20	0.25	0.71**	0.83**
	P*r*							0.71**	0.79**	0.18	0.25	0.70**	0.83**
PH	G*r*								0.56**	0.63**	0.62**	0.76**	0.93**
	P*r*								0.56**	0.62**	0.61**	0.76**	0.92**
TL	G*r*									0.05	0.18	0.24	0.52**
	P*r*									0.036	0.18	0.23	0.51**
CL "a"	G*r*										0.99**	0.67**	0.43*
	P*r*										0.97**	0.66**	0.42*
CL "b"	G*r*											0.62**	0.39*
	P*r*											0.61**	0.38
CR	G*r*												0.84**
	P*r*												0.85**

Note: Plant height (PH), Days to tasselling (DT), Days to silking (DS), Tassel length (LT), Leaves per plant (LPP), Cobs per plant (CPP), Cob length (CL), Grains per cob (GPC), Days to complete maturity (DM), Leaf area (LA), Chlorophyll a (CL "a"), Chlorophyll b (CL "b") and Carotenoids (CR)

Table 5: Direct and indirect effects of different yield components on grain yield and their correlation coefficient

	PH	**DT**	**DS**	**LT**	**LPP**	**CPP**	**CL**	**DM**	**LA**	**CL "a"**	**CL "b"**	**CR**	
Direct Effects	0.492	0.008	-0.653	0.294	0.240	0.133	0.174	-0.476	0.153	0.055	0.0325	0.104	
	Indirect path coefficients												
Path association	**PH**	**DT**	**DS**	**LT**	**LPP**	**CPP**	**CL**	**DM**	**LA**	**CL "a"**	**CL "b"**	**CR**	Correlation coefficient
PH		0.007			0.111	0.037	0.009	-0.013	-0.043	0.009	0.007	-0.656	0.925
DT	0.103		-0.057	0.102	0.019	0.0541	-0.412	0.649	0.112	0.029	0.0007	0.350	-0.961
DS	0.037	-0.025	0.647	-0.092	0.109	-0.391	0.001	-0.112	0.120	-0.676	0.204	0.139	-0.872
LT	0.00008	0.094	-0.097	0.153	0.007	0.1050	0.009	-0.124	-0.046	0.009	-0.461	0.002	0.519
LPP	-0.823	-0.560	-0.875	0.139		0.0093	-0.654	-0.009	0.104	0.127	0.111	0.571	0.833
CPP	0.433	-0.054	0.0009	0.038	0.013		0.742	-0.009	0.095	0.103	0.039	0.149	0.297
CL	0.014	-0.048	0.348	0.079	0.013	0.0394		-0.019	0.024	0.093	0.100	0.123	0.759
DM	0.139	-0.236	0.563	0.008	0.121	0.0009	0.230	0.013		0.543	0.007	0.033	-0.933
LA	0.468	0.003	-0.075	0.101	-0.029	0.1033	-0.009	-0.716		0.094	0.009	-0.541	0.619
CL "a"	0.039	-0.033	-0.312	-0.566	0.024	0.039	0.218	-0.009	0.017		0.055	0.471	0.427
CL "b"	0.127	-0.096	0.007	0.236	0.101	0.114	0.248	-0.006	0.049	0.008		0.104	0.390
CR	-0.106	-0.021	-0.056	0.026	0.103	0.054	0.203	-0.086	0.019	0.033	-0.009		0.849

Note: Plant height (PH), Days to tasselling (DT), Days to silking (DS), Tassel length (LT), Leaves per plant (LPP), Cobs per plant (CPP), Cob length (CL), Grains per cob (GPC), Days to complete maturity (DM), Leaf area (LA), Chlorophyll a (CL "a"), Chlorophyll b (CL "b") and Carotenoids (CR)