Physiological studies of radiation-induced mutants in gram

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INDUCING MUTATIONS by artificial means like irradiation or mutagans results in wider frequency of variability which helps a breeder to build up a raw material for evolutionary improvement of economic crops. Pre-sowing irradiation treatments result mostly in initial shifts in enzymatic activity and metabolic events which in turn result in increase in quality and yield of the crop Studies on physiologicial and biochemical aspects of irradiated progeny helped in evoking a new line of approach in tackling the problems of crop improvement. This basic information will serve as an indices in selecting good ideotypes for further breeding programme, and also will help to retain apparently undersirable types having desirable characteristics as well. The physiological basis of increase in desirable characteristics due to effects of irradiation remains to be investigated in most of the economic crops in view of above the studies were carried out to know the changes in physiological and growth components of six gram mutants as affected by gamma irradiation in RT generation.

MATERIALS AND METHODS

Air dried seeds of gram varities viz. N—59, N—31, Chaffa and Dacca were treated with 15, 30 and 45 KR doses of gamma irradiation in September, 1965. Few new morphological distinct mutants were isolated in R_3 generation. True breeding nature of mutants was isolated in R and R_3 generation and was confirmed in R_4 to R_7 generations. Six mutants, viz. N—59, big leaf, N—31, very big leaf, Chaffa, big pod, Dacca bipinnate, Dacca elongated and Dacca white flower were isolated from irradiated progeny of 15 KR 30, 40, 15, 30 and 15 KR doses respectively, the seeds of which were sown in 1971—72 during *rabi* season along with their respective controls in single rows. The

observations with respect to stomatal frequency, index, length of leaf epidermal hair, maximum leaf thickness, leaf water content, leaf chlorophyll content and leaf respiration were recorded under laboratory conditions. Stomatal number was counted from four miscroscopic fields and average was recorded. The rate of respiration of leaf samples from growing region was determined with Pittenkofer's continuous gas current flow method (Mayer *et al.*, 1955) and expressed as mg. C_2 10 gm/hr at 26° C temperature. For estimation of crude protein content of seed, the ground seed sample was analysed in triplicate, for nitrogen content and percentage nitrogen was multiplied by 6.25 factor. The total chlorophyll content was estimated by colorimetric proredure for pigment analysis (Snell & Snell, 1954).

During the year 1972-73, a replicated progeny row trial of irradiated gram mutants along with their respective controls was laid out in simple R. B. D. in order to know the changes in growth components such as 100 seed weight, number of seeds per plant that ultimately resulted in alteration of yield of gram mutants. Spacing was kept as 30 × 15 cm. The net plot size was 6.0 × 3.0 m. Two rows of each mutant along with checks were sown. At the time of harvest, the yield per plant (average of 5 plants) obtained from four replications was statistically analysed.

TABLE I—Effect of Gamm Irradiation on leaf structure of Gram Mutants

Particulars	*Gram Mutants*						
	N-59	*N*-31	*Chaffa*	*Dacca*	*Dacca Bipinnate*	*Dacca Elongated*	*Dacca White flower*
	Stomatal Frequency/cm²						
Control ..	45455	39773	34091	62501	—	—	—
Mutant ..	34091	34091	39773	—	62501	28409	30491
	Stomatal Index						
Control ..	12·03	11·40	11·73	15·55	—	—	—
Mutant ..	12·79	15·50	12·08	—	7·69	15·71	15·66
	Length of leaf Epidermal hair mm.						
Control ..	0·382	0·253	0·309	0·300	—	—	—
Mutant ..	0·253	0·284	0·213	—	0·359	0·293	0·303
	Maximum leaf thickness in mm.						
Control ..	0·437	0·362	0·546	0·550	—	—	—
Mutant ..	0·508	0·466	0·562	—	0·437	0·450	0·470
	Leaflet Area mm²						
Control ..	36	135	24	24	—	—	—
Mutant ..	160	170	50	—	10	30	32

RESULTS AND DISCUSSION

The variability in phyisological components of gram mutants influenced by gamma irradiation are summarized in Table I, II & III.

I *Leaf structure :* The relevant data are given in Table I.

(1) *Stomatal frequancy and Index :* The data reveal that stomatal frequency is decreased in all the mutants except in chaffa big pod mutant where increase is noted by 14.28% as compared to the control. No change in stomatal number in Dacca bipinnate mutant was seen. Similarly stomatal index is increased in all mutants except in Dacca bipinnate where it is reduced by 7.86% over control. Roy and Clark (1970) also reported significant increase in stomatal index in X-ray irradiated plants of *Vicia faba.*

(2) *Length of leaf epidermal hair :* The length of leaf epidemal hair has been reduced in mutants of N-59, Chaffa big pod and Dacca elongated but it is highly increased in N-31 and daca bipinnate. A significant reduction in length and density of leaf hair in two wheat species reported by Kapoor *et al.* (1965) supports the above finding. Hairy leaf helps in better foliar absorption of minerals and water. In addition, developed leaf hair induces the tolerance to certain pests. Increase in length of leaf hair recorded in mutants of N-31 very big leaf and Dacca bipinnate by 0.031 and 0.059 mm. respectively may be useful for foliar absorption.

(3) *Leaf thicknes sand leaf area* : It is obvious from the data that the leaf thickness is increased in mutants of N-59, N-31 and Chaffa but decreased in all the three mutants of Dacca. The increase ranged from 0.016 to 0.104 mm. as compared to their respective controls. Similarly the findings of Vlasynk and Sil'chenko (1964) as regards increase in size and volume of cells of mesophyll of sugar-beet and fibre flax due to X-ray treatment support the results of present investigations. They further observed that insufficiently utilized assimilates retained in mesophyll cells increased thickness causing diminished yield. However, in present studies increase in thickness of leaf blade in general increased the yield of gram mutants.

II *Physiological studies* : The data on these pertinant observations are presented in Table II.

TABLE II—Physiological studies of radiation-induceed mutants in Gram

Particulars	*Gram Mutants*						
	N-59	*N*-31	*Chaffa*	*Dacca*	*Dacca Bipinnate*	*Dacca Elongated*	*Dacca White flower*
			Leaf Respiration mg CO_2/10 gm/hr				
Control	6·16	3·08	5·72	6·16	—	—	—
Mutant	7·04	4·84	6·60	—	7·48	6·16	7·04
			Leaf Chlorophyll content (% Absorbance)				
Control	58	204	203	102	—	—	—
Mutant	208	222	112	—	90	187	201
			Per cent Crude Protein content of seed				
Control	21·58	23·80	—	21·00	—	—	—
Mutant	22·29	28·00	—	—	19·25	26·25	20·56
			Percent leaf water content				
Control	57·50	57·24	54·07	60·34	—	—	—
Mutant	59·10	58·07	64·71	—	60·00	61·66	46·11
			Colour of chlorophyll Extract				
Control	DY	FG	DG	YR	—	—	—
Mutant	DG	DG	YG	—	Yellow	FG	DY

DY=Deep Yellow ; DG=Dark Green ; FG=Faint Green ; YR=Yellowish Red ; YG=Yellow Green.

(1) *Leaf respiration :* The data indicate that the rate of leaf respiration increased in all the mutants as compared to their respective controls except in Dacca elongated mutant where no change was seen. Bogdashevskaya *et al.* (1967) also reported reduction in leaf respiration in winter rye at 2.5 and 5 KR 'Y' rays irradiation. Such an effect would be beneficial in increasing net grain of dry matter. Increased respiration in present studies suggests that the gamma irradiation resulted in an increase in initial shifts of enzymatic activity.

(2) *Leaf chlorophyl content :* Alteration of chlorophyll content of leaf affected by irradiation treatment is of common occurrence. The observation on chlorophyll content revealed that gamma irradiation considerably increased the chlorophyll content of leaf in mutants of N-59, N-31, Decca elongated and Dacca white flower, while in Chaffa

big pod and Dacca bipinnate ; it is found to be reduced. Pre-planting irradiation treatment also increased chlorophyll content in spring rye at low doses of X-ray (Bogdashevskaya and Runova, 1966) as well as in *Phaseolus vulgaris* (Sucin and Henegarin, 1967). However, reduction in chlorophyll content is also reported by Roy and Clark (1970) due to X-ray in *Vicia faba.*

It appears from the colours of the chlorophyll extract that the mutants of Chaffa, Dacca bipinnate and Dacca white flower contains more proportion of carotenoids and xanthophyll as compared with the chlorophyll a and b. As increase in chlorophyll content stimulate the food synthesis, this change may have significance in altering yield. However, Tai and Todd (1972) observed reduction in carotenoid contents of Lutescens mutants in ground nut as compared to those of normal in their chromatographic studies of pigment analysis.

(3) *Leaf water content :* The water content of the leaves is increased in all the mutants and ranged from 0.34 to 10.64% except in mutants of Dacca elongated and Dacca white flower. Increased water content results in maintaining the plant protoplasm always at high water potential.

(4) *Crude protein content in seed :* Negative as well a positive effect of irradiation regarding change in CPC are reported by many workers. Virughese and Swaminathan (1967) reported 2.5% increase in protein content in Sarbati-Sonora wheat as compared to Sonora-64 due to gamma ray treatment. Uprety (1968) also observed that 4 KR dose of gamma rays increased the protein content in *Vigna unguiculata.* In present studies also appreciable increase in CPC to the extent of 4.20 and 5.25% over control is observed in N-31 very big leaf and Dacca elongated mutants respectively. Very big leaf mutant of N-31 not only yielded 48.38% more over control but also found to contain 4.20% more protein in its grain and therefore, its cultivation will offer substantial gain to a farmer with respect to yield as well as protein. In a country like India where millions are suffering from malnutrition, such a type of mutant will certainly help to cater the needs of masses if cultivated on large scale. In addition, N-31 is early and hence it may stand better significance.

III *Growth components.*—The observations recorded on effect of gamma irradiation in trial of 1972-73 are summarized in Table—III and also graphically presented in figure 1.

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TABLE III—Effect of Gamma Irradiation on Growth Components of Gram Mutants

Particulars	*Gram Mutants*							
	N-59	*N*-31	*Chaffa*	*Dacca*	*Dacca Bipinnate*	*Dacca Elongated*	*Dacca White flower*	*Remarks*
				Number of seeds per plant				
Control ..	33·12..	14·81..	32·00..	43·57..	— ..	— ..	— ..	SE=4·37
Mutant ..	25·56..	24·37..	35·75..	— ..	44·62..	38·69..	56·25*..	CD=12 68
Percent increase/ decrease..	—22·82..	+64·55..	+11·71..	— ..	— 2·88..	—10·79	+29·69 ..	—
				100 *Seed weight in gram*				
Control ..	37·0 ..	37·0 ..	12·0 ..	15·0 ..	— ..	— ..	— ..	—
Mutant ..	28·0 ..	35·0 ..	22·0 ..	— ..	10·0 ..	12·0 ..	14·0 ..	—
				Mean Seed Yield per plant gm.				
Control ..	5·10..	4·65..	5·40..	5·60..	— ..	— ..	— ..	NS
Mutant ..	5·50..	6·90..	6·30..	— ..	3·95..	3·60 ..	6·05 ..	SE—0·866
Percent increase/ decrease..	+ 7·84..	+48·38..	+16·66..	— ..	—29·46..	—25·71	+ 8·03 ..	—

* Significant at 5% level. NS=Non-significant.

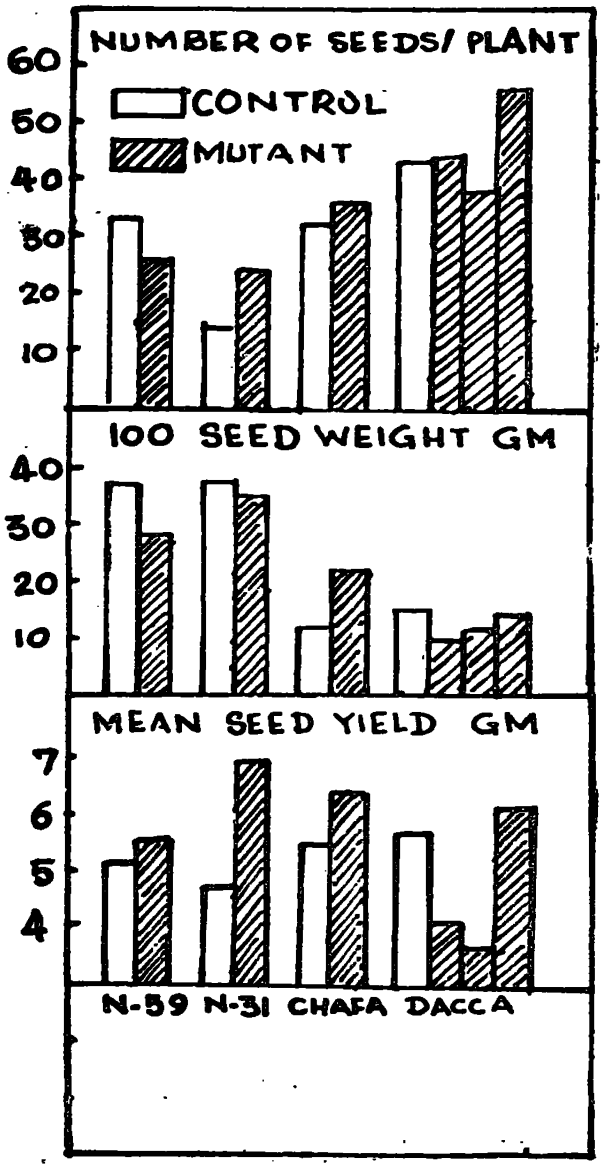
(1) *Number of seeds per plant.*—It is clear from the data that in case in Dacca white flower mutant there is only significant increase in number of seeds per plant as compared to its control, but in other mutants differences are non-significant. However, in general, increase in number of seeds per plant also correspondingly increased the grain yield of some mutants of gram. Though number of seeds of N-59 big leaf mutant is decreased by 22.82% the increase in yield is found to be 7.84% over control. This may be attributed to the maximum increase in leaf area (34.4%) followed by exceptional increase in chlorophyll content as well as leaf thickness.

In contrast to above increase in number of seeds by 2.88% resulted in reduction in yield by 29.46% in case of Decca bipinnate. This may perhaps be due to reduced leaf area and low chlorophyll content.

(2) *100 seed weight.*—The data reveals that 100 seed weight is highly reduced in most of the mutants as compared to their respective controls, Chaffa mutants bear big pods where they are greatly

increased. This shows that grain filling of Chaffa mutant is considerably increased which may be due to better translocation of photosynthates to the grain, Amer and Hakeem (1964) also reported slight decrease in 100 seed weight of *Lupinus termis* when it was treated with 4 to 12 KR and 20 KR gamma rays.

FIGURE-1



(3) *Mean seed yield.*—Statistical analysis revealed that the yield differences are not significant. However, marked increased in mean seed yield of gram to the extent of 7.84, 48.38, 16.66 and 8.03% over controls is observed in mutants of N-59 big leaf, N-31 very big leaf, Chaffa big pod and Dacca white flower. Both Dacca elongated and hipinnate yielded considerably less than their respective controls, the reduction being 25.71 and 29.46% respectively. This may be attributed

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to the thinness of leaf and greater proportion of yellow pigments in leaves. The increase in yield is also reported in potato (Berezina *et al.*, 1963) and castor (Shivraj and Raman rao, 1963). However, reduction in yield due to radiation has not been uncommon as reported in pea by Tselishchev *et al.* (1965) and in groundnut by Sanjeeviah *et al.* (1967) as well.

SUMMARY

Investigations were carried out at College of Agriculture, Parbhani, during 1971/72 and 72-73 to study the variation in physiology and growth components of six morphologically distinct mutants in gram, isolated from gamma irradiated progenies. Reduced leaf area and stomatal index followed by increased leaf hair was observed in Dacca bipnnate mutant. All the mutants retained more moisture in their foliages. Leaf thickness increased in N-59, N-31 and Chaffs mutants while it decreased in all the three mutants of Dacca. Leaf respiration was more in the mutants. Cholorophyll content increased in four mutants but decreased in two, N-31—very big leaf mutant not only out-yielded the control by 48.30% but contained 4.20% more protein in its grain. Dacca elongated had also 5.25% more protein but yielded less. Chaffa big pod mutant yielded 16.66% more than control The number of seeds significantly increased in Dacca white flower with increase in yield by 80.3%.

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* Original not seen.