Effect of Gamma Irradiatoin on Chlorophyll Metabolism in Dolichos, Vigna and Phaseolus species¹

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Different plants, with special reference to their chlorophyll metabolism, have been studied by different workers such as Arnon (1949), Aruga and Monsi (1963), Anderson and Boardman (1964), Wood and Bachelor (1969), Gopal Rao (1973) Katyayani *et al.* (1978).

Dolichos, Vigna and *Phaseolus*, the members of family Papilionaceae, are mainly grown for their beans which are used as vegetables and pulses. It is well known to the biologists that some bacteria, in symbiotic association with leguminous plants, fix atmospheric nitrogen which leads to the formation of amino acids and proteins. These products are translocated to the seeds during the period of their formation. Due to this, the seeds of the leguminous plants contain higher amount of protein in comparison to the seeds of other plants.

The mechanism involved in the nitrogen fixation indicates that its efficiency is regulated by the supply of the host photosynthate.

Therefore, induction of higher amount of chlorophyll may affect the photosynthetic activity of the plant, which in turn, may affect the yield of the plant concerned.

Keeping this fact in mind, the present piece of work has been carried out to see the effect of acute gamma radiation on the chlorophyll metabolism of the plants concerned, and their yield at corresponding doses.

Materials and methods

Pure line seeds of *Dolichos*, *Vigna* and *Phaseolus* were exposed to 1.25 KR, 2.5 KR, 5.0 KR, 10.0 KR and 15.0 KR doses of gamma irradiation from Co⁸⁰ source. Plants were grown from these seeds and control plants were maintained in each case. Chlorophyll a, chlorophyll b and total chlorophyll content was estimated in the leaf of all the samples and the effect of gamma rays on chlorophyll metabolism was measured in terms of mg/gm fresh weight of leaf.

Chlorophyll content in terms of mg/gm fresh weight of leaf was calculated by the method suggested by Arnon (1949), Anderson and Boardman (1964) and Wood and Bachelord (1969). Care was taken in selecting leaf materials of the same age (4 weeks after sowing) randomly from different plants.

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Observations and results

Chlorophyll b was recorded to be more in amount than chlorophyll a in all the cases investigated.

In *Dolichos lablab* Linn., total chlorophyll content decreased at lower doses upto 5 KR but increased at 10 KR and 15 KR when compared to chlorophyll content of control plants. The maximum amount (0.585 mg/gm) was noted at 15 KR dose. The total chlorophyll content in control plants was recorded to be 0.497 mg/gm (Table 1, Graph 1). Here, the weight of 100 seeds went on increasing with the increase in the radiation dose.

But *Dolichos biflorus* Linn. proved to be more radiosensitive than the former where an increase in total chlorophyll content was noted at all dose levels. The control value (0.667 mg/gm) was the minimum and the maximum value (0.937 mg/gm) was recorded at 1.25 KR dose. The minimum value among treated plants (0.734 mg/gm) was recorded at 10 KR dose level (Table 1, Graph 2). However, a linearity existed as regards the radiation dose and the weight of 100 seeds.

Plant name	Dose in KR	Chl. a mg/gm	Chl. b mg/gm	Total chl. mg/gm	Weight o 100 seeds in grams
Dolichos lablab	0	0.175	0.322	0.497	10.642
var. 3/76	1.25	0.176	0.319	0.495	11.675
	2.5	0.155	0.277	0.432	11.692
	5.0	0.151	0.296	0.447	12.698
	10.0	0.197	0.372	0.569	14.714
	15.0	0.204	0.381	0.585	14.721
Dolichos biflorus	0	0.241	0.426	0.667	2.947
var. local	1.25	0.325	0.612	0.937	3.268
	2.5	0.278	0.497	0.775	3.387
	5.0	0.291	0.494	0.785	3.494
	10.0	0.249	0.485	0.734	3.612
	15.0	0.298	0.494	0.792	3.719

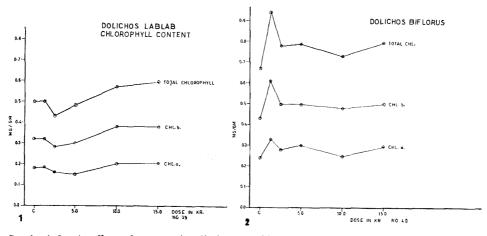
 Table 1. Effect of gamma irradiation on chlorophyll content of leaf in two species of *Dolichos* in M₁ generation

Out of the three varieites of Vigna unguiculata (L.) Walp subspecies cylindrica, the variety Rituraj showed a negative trend in chlorophyll metabolism. The control value (0.567 mg/gm) was the maximum value. The minimum value (0.378 mg/gm) was noted at 1.25 KR dose and the maximum value among the irradiated population (0.541 mg/gm) was recorded at 10 KR dose level. In this variety, radiation seemed to retard the chlorophyll metabolism. The decrease in value of total chlorophyll was more at lower dose levels than at the higher ones (Table 2, Graph 5).

A somewhat similar trend was recorded in the variety Pusa Dofasli of the same species. All the lower doses decreased the amount of chlorophyll as compared to that in control plants (0.546 mg/gm) but there was an increase in chlorophyll content at 15 KR dose where it was noted as 0.596 mg/gm. The minimum value (0.493

mg/gm) was recorded at 2.5 KR dose levlel (Table 2, Graph 3).

In the variety Pusa Falguni of the same species, a decrease in chlorophyll content was recorded at lower doses (1.25 KR and 5 KR) but an increase was noted at



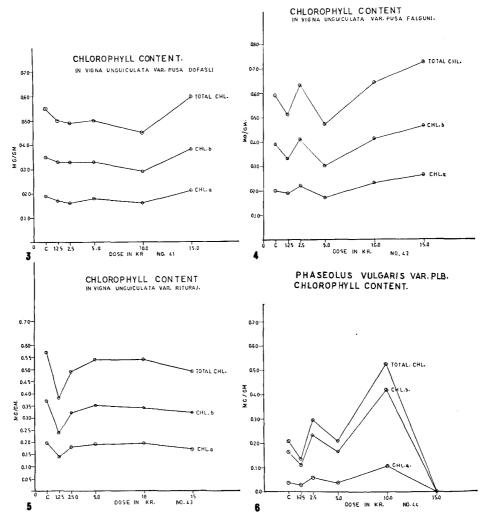
Graphs 1-2. 1, effect of gamma irradiation on chlorophyll metabolism in leaves of *Dolichos lablab* L. 2, effect of gamma irradiation on chlorophyll metabolism in leaves of *Dolichos biflorus* L.

Name of variety	Dose in KR	Chl. a mg/gm	Chl. b mg/gm	Total chl. mg/gm	Weight o 100 seeds in grams
Pusa Dofasli	0	0.194	0.352	0.546	7.6
	1.25	0.172	0.328	0.500	6.3
	2.5	0.163	0.330	0.493	7.1
	5.0	0.181	0.326	0.507	7.9
	10.0	0.160	0.288	0.448	7.9
	15.0	0.214	0.382	0.596	14.7
Pusa Falguni	0	0.203	0.390	0.593	6.4
	1.25	0.185	0.327	0.512	6.6
	2.5	0.220	0.411	0.631	6.7
	5.0	0.167	0.304	0.471	6.7
	10.0	0.230	0.414	0.644	5.7
	15.0	0.258	0.461	0.719	5.6
Rituraj	0	0.195	0.372	0.567	7.4
	1.25	0.135	0.243	0.378	7.8
	2.5	0.167	0.318	0.485	8.2
	5.0	0.193	0.346	0.539	8.1
	10.0	0.198	0.343	0.541	6.0
	15.0	0.173	0.319	0.492	5.8

Table 2. Effect of gamma irradiation on chlorophyll content of leaf in three varieites of *Vigna unguiculata* (L.) Walf subspecies *cylindrica* in M₁ generation

2.5 KR, 10 KR and 15 KR dose levels. The maximum value (0.719 mg/gm) was recorded at 15 KR dose and the minimum (0.471 mg/gm) at 5 KR dose as compared with control value of 0.593 mg/gm (Table 2, Graph 4). All the three varieites

of V. unguiculata responded differently to the radiation dose, as regards the weight of the equal number of seeds. In the variety Pusa Dofasli, the lower doses seemed to be inhibitory while at higher doses, the seed weight increased.

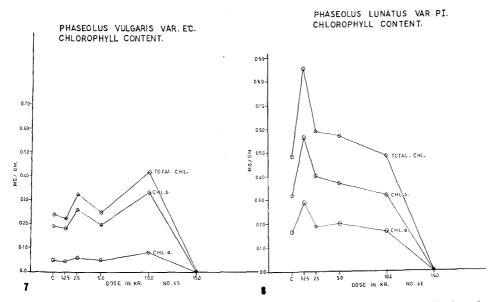


Graphs 3-6. 3, effect of gamma irradiation on chlorophyll metabolism in leaves of Vigna unguiculata (L) Walp subspecies cylindrica var. Pusa Dofasli. 4, effect of gamma irradiation on chlorophyll metabolism in leaves of Vigna unguiculata (L) Walp subspecies cylindrica var. Pusa Falguni. 5, effect of gamma irradiation on chlorophyll metabolism in leaves of Vigna unguiculata (L) Walp subspecies cylindrica var. Rituraj. 6, effect of gamma irradiation on chlorophyll metabolism in leaves of Phaseolus vulagris L, var. PLB.

In Pusa Falguni, the higher doses (10 KR and 15 KR) registered inhibitory effect while the lower doses induced some positive results. In the variety R. R., higher values of seed weight was observed at 1.25 KR and 5 KR. But higher and lower values of this dose proved to be inhibitory.

Name of species and variety	Dose in KR	Chl. a mg/gm	Chl. b mg/gm	Total chl. mg/gm	Weight of 100 seeds in gram
Phasolus vulgaris L.	0	0.043	0.165	0.208	26.6
variety PLB	1.25	0.028	0.109	0.137	33.4
	2.5	0.060	0.237	0.297	33.4
	5.0	0.043	0.164	0.207	33.1
	10.0	0.107	0.419	0.526	27.5
P. vulgaris L.	0	0.050	0.190	0.240	34.6
variety EC.	1.25	0.044	0.179	0.223	35.3
	2.5	0.062	0.261	0.323	37.3
	5.0	0.051	0.197	0.248	37.3
	10.0	0.085	0.336	0.421	37.3
P. lunatus L.	0	0.165	0.319	0.484	29.0
variety PI	1.25	0.292	0.561	0.853	31.5
·	2.5	0.189	0.402	0.591	28.7
	5.0	0.201	0.371	0.572	15.5
	10.0	0.163	0.322	0.485	41.0
P. lunatus L.	0	0.183	0.365	0.548	24.4
variety EC	1.25	0.179	0.339	0.518	25.5
	2.5	0.169	0.332	0.501	28.7
	5.0	0.130	0.254	0.384	23.6
	10.0	0.108	0.238	0.346	25.8
	15.0	0.085	0.219	0.304	28.9

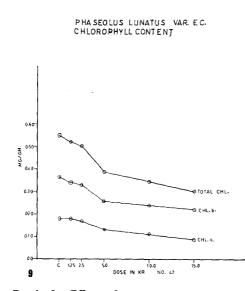
Table 3. Effect of gamma irradiation on chlorphyll content of leaf in four variettes belonging to two species of *Phaseolus* in M_1 generation



Graphs 7-8. 7, effect of gamma irradiation on chlorophyll metabolism in leaves of *Phaseolus* vulgaris L. var. EC. 8, effect of gamma irradiation on chlorophyll metabolism in leaves of *Phaseolus* lunatus L. var. PI.

In *Phaseolus vulgaris* Linn., both the varieties PLB and EC exhibited a decrease at lower dose levels and increase at higher dose levels as compared to the control value.

In variety PLB, total chlorophyll in control plants was recorded as 0.208 mg/gm. The mxaimum value (0.526 mg/gm) was noted at 10 KR dose and the minimum (0.137 mg/gm) was recorded at 1.25 KR dose. The plants at 2.5 KR dose level exhibited an increse (0.297 mg/gm) while those at 5 KR dose level recorded a slight decreae (0.207 mg/gm) (Table 3, Graph 6).



Graph. 9. Effect of gamma irradiation on chlorophyll metabolism in leaves of *Phaseolus lunatus* L. var. EC.

In variety EC of the same species, the minimum value (0.223 mg/gm) was recorded at 1.25 KR dose as compared to control value of 0.240 mg/gm. All other treatments showed incresae in chlorophyll content, the maximum (0.421 mg/gm) being recorded at 10 KR dose level. The variety EC seemed to be more radiosensitive than variety PLB. In both the varieties, a tendency of decrease in mutagenic effect was observed at 5 KR dose level after a significant increase at 2.5 KR dose. The dose 1.25 KR seemed to be detrimental to chlorophyll metabolism while 10 KR dose appeared to be the greatest stimulant in this respect (Table 3, Graph 7).

In *P. lunatus* Linn., the variety PI seemed to be positively radiosensi-

tive and the variety EC negatively so. In the former, an incresse in chlorophyll content over the control value of 0.484 mg/gm was noted at all dose levels. The maximum value (0.853 mg/gm) was observed at 1.25 KR dose and the minimum (0.485 mg/gm) at 10 KR dose level. The positive trend towards increase in chlorophyll content gradually declined from 1.25 KR to 10 KR dose level (Table 3, Graph 8).

In contrast, there seemed to be a gradual and uniform decrease in chlorophyll content in irradiated population as compared to the control value of 0.548 mg/gm in the variety EC of the same species. The minimum value (0.304 mg/gm) was recorded at 15 KR dose. The lower doses proved less detrimental than higher doses to chlorophyll metabolism (Table 3, Graph 9).

In *P. vulgaris*, the seed weight in all the treated populations increases in comparison to the control plant. But at 10 KR dose the seed weight decreased, which was, however, higher than that of the control plants.

In *P. vulgaris* var. EC, the seed weight increased upto 2.5 KR dose, the value remained constant even at 10 KR dose. In both the varieties of *P. lunatus* (viz. PI and EC), the seed weight did not show any correlation with the radiation dose

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(Table 3).

Discussion

The effect of gamma rays on chlorophyll metabolism was measured in terms of chlorophyll content mg/gm fresh weight of leaf. The data presented in Tables 1 to 3 and Graphs 1–9 showed that 10 KR and 15 KR doses caused an increase in the content of chlorophyll a, chlorophyll b and total cholrophyll in *Dolichos lablab* as compared to that in control leaf. It caused a decrease in chlorophyll content at 1.25 KR, 2.5 KR and 5 KR doses. The maximum increase and decrease were noted at 15 KR dose and 2.5 KR dose respectively (Table 1).

In *D. biflorus*, the gamma rays caused as increase in chlorophyll content at all the dose levels over that of control, the maximum increase being observed at 1.25 KR dose (Table 1).

In Vigna unguiculata variety Pusa Dofasli, the radiation caused an increase at 15 KR dose and decrease at all other doses.

The variety Pusa Falguni of the same species recorded an increase at 2.5 KR, 10 KR and 15 KR dose levels, the maximum increase being recorded at 15 KR dose.

But the variety Rituraj of *Vigna* recorded only decrease in chlorophyll content at all dose levels as compared to that of control (Table 2).

The variety PLB of *Phaseolus vulgaris*, recorded incresae in chlorophyll content at 2.5 KR and 10 KR dose levels whereas the var. EC of the same species exhibited an increase at 2.5 KR, 5 KR and 10 KR dose levels. The maximum increase in both the varieties was noted at 10 KR dose level (Table 3).

The variety PI of P. *lunatus* recorded an increase in chlorophyll content over that of control at all dose levels, the maximum increase being noted at 1.25 KR dose level. In contrast to this, gamma rays appeared to have inhibited the rate of chlorophyll metabolism in the variety EC of the same species at all dose levels as compared to that in control plants (Table 3).

The 10 KR and 15 KR doses seemed to cause greatest stimulation to chlorophyll metabolism in five out of nine types, while 1.25 KR dose produced the greateast stimulation in two of them. In the remaining two types, radiation caused decrease in chlorophyll content at all dose levels.

Similar observations were made by Katyayani *et al.* (1978) in *Phaseolus aureus*, Gopal Rao (1973) in the same species when treated with riboflavin, and Rao and Rao (unpublished) in Okra with gamma rays. They had reported increase in chlorophyll metabolism at higher doses of gamma rays (8 to 10 KR) and decrease in the content at lower doses (5 to 6 KR).

An increase in chlorophyll content at 1.25 KR dose in *P. lunatus* variety PI and in *Dolichos biflorus* in contrast to other five varieties and species in which lower doses decreased the content can be explained on the basis that the cytological and genetic make up of these two types was different and hence it had differential response to different doses of irradiation.

In the variety Pusa Dofasli of Vigna unguiculata, 100 seeds weight was almost double at 15 KR dose level as compared to other doses. This might be attributed

to the fact that radiation caused greater sterility at this highest dose level, so less number of seeds were formed per pod, which led to the increase in size of the seeds. Thereby the 100 seeds weight might have shown an apparent increase as compared to the control.

In the variety PI of *Phaseolus lunatus*, 100 seeds weight was almost half at 5 KR dose than that at other doses. This might be due to the fact that the number of seeds per plant as well as the number of pods per plant were the greatest at this dose, so the seeds formed were smaller in size. Thus the 100 seeds weight might have shown an appreciable apparent decrease over those of control plants and treated plants at other doses.

The dose 15 KR appeared to be most damaging for PLB and EC varieties of *Phaseolus vulgaris* and the variety PI of *Phaseolus lunatus* whereas no such phenomenon was noted in other species and varieties. The plants did not survive at this dose level beyond the 20th day. This might be due to the fact that the above noted three varieties were more susceptible to the damages caused by radiation than other varieties and species under investigation. The death might have occurred due to agromomic reasons also. This was applicable to control plants as well.

Although a clear picture does not exist in all the treated plants as regards their response to the radiation dose, in terms of the seed weight and their correlation with chlorophyll content, it is quite apparent that the chlorophyll content has got a role to play in determining the weight of the seeds. In *Dolichos lablab* and *D. biflorus* the chlorophyll content and seed weight registered a linear relationship. But the species and varieties of *Vigna* and *Phaseolus* did not show such trend. This may be due to their different radiosensitive nature. However, it needs further investigation.

Abstract

The effect of gamma irradiation on chlorophyll metabolism was studied in leaves of *Dolichos, Vigna* and *Phaseolus* species. The dry seeds were exposed to 1.25 KR, 2.5 KR, 5.0 KR, 10.0 KR and 15.0 KR doses of gamma irradiation and leaves from plants recovered from them as well as from control plants were tested for Chl. a, Chl. b and total chlorophyll contents. A comparison was made between chlorophyll contents of leaves from irradiated and non-irradiated populations. Chlorophyll content decreased at lower doses and incerased at higher doses in *Dolichos lablab* L., *Vigna unguiculata* L. and *Phaseolus vulgaris* L. But in *D. biflorus* and *P. lunatus* L. var. PI irradiation increased the chlorophyll content at all dose levels. In contrast the variety EC of *P. lunatus* L. recorded a decrease in chlorophyll content at all dose levels. The amount of chlorophyll b was more than that of chlorophyll a in all the plants investigated.

References

- Anderson and Boardman, 1964. In C.P. Malik's Estimation of Chlorophyll Content Plant Physiology Vikas Publications, Delhi.
- Arnon, D. I. 1949. Copper enzymes in isolated chloroplasts, polyphenol oxidase in *Beta vulgaris*. Plant Physiol. 24: 1-15.

- Aruga, Y. and Monsi, M. 1963. Cholorophyll amounts as an indicator of matter production in bio-communities. Plant and Cell Physiol. 4: 29-39.
- Gopal Rao, P. 1973. Influence of riboflavin on growth, respiration and chlorophyll content and protein content in green gram (*Phaseolus radiatus* Linn). Curr. Sci. **42**: 580-581.
- Katyayani, M., Rao, D., Rao, S. N. and Krishnamurty, P. 1978. Gamma irradiation induced physiological variabilities in *Phaseolus aureus* Roxb. J.I.B.S. 57 (Suppl.): 91 (abstract).
- and Rao, D. 1980. Variability and radio-sensitivity of Vigna radiata (L.) Wilcez. var. Baisakhi as affected by gamma rays. J.I.B.S. 59 (Suppl): 116 (abstract).
- Kachroo, P. 1970. Pulse Crops of India I.C.A.R., New Delhi.
- Wood, J. P. and Bachelord, E. P. 1969. Variations in chlorophyll concentration in the foliage of radiata pine. Forestry: 33: 9-14.
