STIMULATION OF GROWTH IN *OCIMUM KILIMANDSCHARICUM* BY LOW-DOSE X-IRRADIATION

By B. JOSEPH,* B. K. GAUR,* M. S. CHADHA,† and A. V. PATANKAR†

[Manuscript received 11 October 1972]

Abstract

The effect of low-dose X-irradiation (125–2500 R) of *O. kilimandscharicum* seeds on germination, growth, and development was studied. Exposure to 1500 and 2500 R brought about marked stimulation in growth and flowering. The increase in foliar growth has resulted in a higher net yield of essential oil and camphor.

I. INTRODUCTION

The effects of ionizing radiation on plant growth has received considerable attention and many studies in plant breeding deal with mutations induced by large doses of acute and chronic radiation (Ehrenberg 1971). However, papers dealing with the stimulating effects of low doses of radiation are limited. Some common effects such as increase in germination rate, shoot and root growth, and early flowering have been reported but the results are often not reproducible and some are contradictory (Johnson 1936; Breslavets 1946; Kuzin 1956; Sax 1955, 1963; Sax and Schairer 1963; Anon. 1966). As there was a lack of information on the effects of low doses of radiation on *Ocimum kilimandscharicum*, the leaves of which are an important source of essential oil rich in camphor, the present investigation was undertaken. The effect of X-irradiation of seeds on subsequent oil and camphor production by the plant was investigated.

II. MATERIALS AND METHODS

Seeds of *O. kilimandscharicum* of water content 4·5% were spread uniformly in a single layer and exposed to X-rays (250 kV potential, 15 mA, aluminium foil) of intensities ranging from 125 to 2500 R at an exposure rate of 200 R per minute.

Within 2 hr of irradiation, both control and irradiated groups of seeds were sown in soil in earthenware pots 30 cm wide by 8 cm high, at 225 seeds per pot. Six-week-old seedlings were transplanted in the field, leaving 25 cm between plants and 60 cm between rows. Using a completely randomized block design, the experiment was replicated four times. Each sample consisted of 10 plants per treatment per replication. The net plot size varied with the number of radiation treatments and the sampling stages in any one experiment.

Germination studies were carried out in the dark at 24°C and 60% R.H. Each treatment was replicated five times using 25 seeds per replicate. The seeds were sown between two layers of moist filter paper and a daily count of germination, as shown by radicle emergence, was made until 112 hr from sowing, at which time all treatments had reached maximum germination.

* Biology and Agriculture Division, Bhabha Atomic Research Centre, Bombay-85, India.
† Bio-Organic Division, Bhabha Atomic Research Centre, Bombay-85, India.

The following observations were made on plants grown in the field:

(1) fresh and dry weights of leaves and stem;
(2) percentage of plants flowering;
(3) total chlorophyll content in leaves;
(4) oil and camphor content in leaves.

Growth observations and chemical estimations were made at intervals of 2 weeks beginning at transplantation; flowering was checked daily till all plants had flowered. Dry weight was determined by drying to a constant weight at 60°C in a forced hot-air oven. Chlorophyll was measured according to the technique of Bruisma (1963) on three samples from each of five plants per treatment. Oil and camphor were determined in two samples of fresh leaves from each of three plants per treatment. Oil was steam-distilled from fresh leaves and estimated gravimetrically. Camphor in the oil was determined quantitatively on an Aerograph model No. A-350-B gas–liquid chromatograph using a 5 ft by ¼ in. column (20% DEGS on fire-brick).

All results were analysed statistically using the *t*-test.

III. Results

The germination rate of control seeds and those irradiated with 500, 1500, and 2500 R is given in Table 1. While treatment with 1500 R improved the rate throughout the germination period, there was no difference from controls for 2500 R irradiation. Seeds irradiated with 500 R showed significantly higher percentage germination 64 hr after sowing. The most effective radiation treatment (1500 R) improved not only the rate but also total germination, which increased by more than 25% at the peak level.

Table 1

PERCENTAGE GERMINATION OF UNIRRADIATED AND IRRADIATED SEEDS OF *O. KILIMANDSCHARICUM*

Values are means of five replicates ± standard error

Radiation dose (R)	Percentage germination at			
	40 hr	64 hr	88 hr	112 hr
0	48 ±3·8	64 ±1·8	66 ±1·9	66 ±2·0
500	56 ±2·3	73* ±2·9	73 ±2·8	74 ±2·8
1500	68* ±1·3	79* ±2·5	83* ±2·3	83* ±2·7
2500	56 ±3·5	61 ±2·5	61 ±2·4	61 ±2·7

* Significantly different from control at 5% level.

Fresh and dry weights of leaves and stem 8, 10, 12, and 14 weeks after sowing are presented in Table 2. Almost all the radiation treatments induced greater leaf growth at some stage. There was a more consistent effect for dry weight than fresh weight, apparently due to differential loss of water from plants during sampling and observation. Dry weight data clearly show that the relative weight gains not only increased with increasing doses but also gradually decreased as the plants aged. In fact, no significant radiation effect was obtained on leaf dry weight after 10 weeks in the 125, 250, and 500 R treatments. Harvest at 12 weeks would give a maximum leaf weight while also allowing most of the advantage of the radiation-induced stimulation (>80% in fresh and 50% in dry weights respectively at 2500 R).

The trends in the radiation effects on stem growth were similar to, if not greater than, those in leaves (Table 2). Except in the 500 R treatment, dry matter accumulation increased generally, though not always significantly, with increasing radiation exposures. As with leaves, the stimulation was relatively greater in younger than older plants.

TABLE 2

FRESH AND DRY WEIGHT OF LEAVES AND STEM PER PLANT FROM UNIRRADIATED AND IRRADIATED *O. KILIMANDSCHARICUM* SEEDS

Values are means of four replicates ± standard error

Age of plants (weeks)	Radiation dose (R)	Fresh weight (g) Leaves	Fresh weight (g) Stem	Dry weight (g) Leaves (A)	Dry weight (g) Stem (B)	Ratio A/B
8	0	1·24 ±0·14	0·40 ±0·04	0·24 ±0·01	0·13 ±0·02	1·9
	125	3·02* ±0·36	0·86* ±0·13	0·48* ±0·01	0·18 ±0·02	2·7
	250	3·04* ±0·42	0·73* ±0·12	0·50* ±0·01	0·20* ±0·01	2·6
	500	4·57* ±0·45	1·55* ±0·02	0·88* ±0·09	0·28* ±0·05	3·1
	1500	6·93* ±0·89	1·95* ±0·28	1·09* ±0·10	0·35* ±0·15	3·1
	2500	13·78* ±1·73	5·03* ±0·55	1·99* ±0·23	0·96* ±0·13	2·1
10	0	41·1 ±1·89	26·1 ±1·67	8·7 ±0·31	8·2 ±0·65	1·7
	125	51·8* ±2·22	42·1* ±2·50	13·5* ±0·91	12·0* ±1·16	1·1
	250	55·7* ±4·85	27·0 ±1·68	15·5* ±2·05	7·7 ±1·04	2·1
	500	53·8* ±4·04	35·1* ±1·89	10·8* ±0·68	7·5 ±0·69	1·4
	1500	47·1 ±3·86	29·4 ±1·03	15·3* ±1·30	10·8 ±0·96	1·4
	2500	50·4* ±1·86	41·7* ±2·74	15·7* ±1·27	13·3* ±1·33	1·2
12	0	61·6 ±5·91	33·6 ±3·29	15·7 ±1·67	14·4 ±1·42	1·1
	125	77·6* ±3·97	41·4 ±2·13	18·6 ±1·02	16·7 ±1·28	1·1
	250	74·7* ±2·18	44·8* ±1·28	18·6 ±1·00	19·1* ±1·15	1·0
	500	66·4 ±3·91	37·2* ±2·20	16·9 ±0·88	14·4 ±1·23	1·2
	1500	106·7* ±3·43	60·4* ±1·42	22·6* ±1·55	20·2* ±1·12	1·1
	2500	113·2* ±4·61	75·9* ±3·04	24·1* ±0·68	25·0* ±1·33	1·0
14	0	105·8 ±4·70	96·3 ±4·64	12·8 ±1·56	15·3 ±1·24	0·8
	125	99·8 ±3·85	87·5 ±2·45	10·7 ±0·99	18·0 ±1·51	0·6
	250	159·5* ±3·14	147·2* ±5·68	16·4 ±1·38	22·6* ±1·31	0·7
	500	185·3* ±7·92	153·0* ±8·04	16·8 ±1·80	15·5 ±1·57	1·1
	1500	117·0 ±3·49	148·8* ±6·01	21·4* ±1·34	26·1* ±2·04	0·8
	2500	121·5* ±3·45	103·2 ±3·92	21·3* ±1·68	33·7* ±3·34	0·6

* Significantly different from control at 5% level.

In order to compare the response to radiation of the leaf and stem, leaf : stem ratios have been calculated on a dry weight basis at different stages of plant growth (Table 2). In both controls and experimental plants these ratios were highest in young plants and decreased with age. This would indicate that as the plants aged, stems grew relatively more than leaves. Radiation increased the leaf : stem ratio, but only till plants were 10 weeks old. This increase was definitely greater in the 250–1500 R range than at higher or lower exposures. Thus radiation generally induced more growth in leaves than in stem, the optimum range being 250–1500 R. A near similarity in the ratios in older plants shows that the differential sensitivity of leaves and stems is limited to early growth only.

Data on flowering behaviour are presented in Figure 1 in terms of percentages of plants flowering at various periods. Early flowering induced by radiation of 125 R and higher doses is apparent at 9 weeks. This behaviour, though less conspicuous at 500 R, was maintained for 4 more weeks, after which all plants, both control and experimental, were flowering. The percentage of plants in flower was about the same at 250, 1500, and 2500 R in plants 10 weeks old and older.

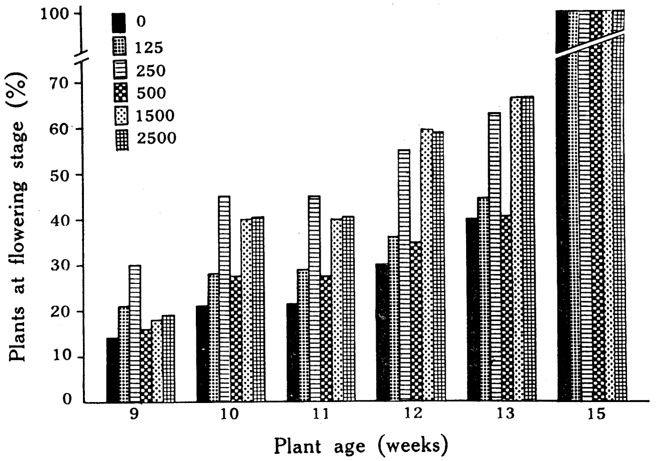

Fig. 1.—Effect of radiation on flowering of *O. kilimandscharicum* plants. Radiation doses (in roentgens) are indicated in the key.

Chemical estimations for chlorophyll, oil, and camphor contents in leaves are given in Table 3. While chlorophyll content per unit fresh weight rose steadily with age in both control and experimental plants, radiation as such did not alter its level significantly. A rise in the chlorophyll content at 1500 R was observed at 10 weeks but not later.

Oil content in leaves 6 and 8 weeks old was little affected, on a fresh weight basis, by seed irradiation (Table 3). All three dose levels caused an increase of nearly 30% in 10-week-old plants, while 2500 R caused a decrease of 15% at 13 weeks.

There was no clear-cut trend in effect of radiation on camphor content of oil (Table 3). However, a small increase in camphor content was observed at 13 weeks in both the 500 and 1500 R treatments, although radiation at these levels was inhibitory at 6 and 8 weeks.

Table 4 summarizes findings from a recent field trial (November 1971–March 1972) in which the effects of radiation doses of 1500 and 2500 R were tested. This experiment consisted of 10 replicates, with 100 plants per treatment per replication. Half the population was harvested at 9 weeks and the rest at 14 weeks. The percentage survival was calculated on the total number of plants obtained from the two harvests out of those transplanted. A highly significant increase in shoot growth (fresh and dry weights) was achieved for both radiation doses when plants were harvested at 9 weeks. The increase of 100–150% in leaf dry weight in the experimental plants

TABLE 3

CHLOROPHYLL, OIL, AND CAMPHOR CONTENTS OF LEAVES OF *O. KILIMANDSCHARICUM* FROM UNIRRADIATED AND IRRADIATED SEEDS

Values are means ± standard error

Parameter	Time (weeks)	Content at radiation dose of: 0	500 R	1500 R	2500 R
Chlorophyll† (mg/2 g fresh wt.)	6	8·8 ±0·65	—	9·8 ±0·27	7·3 ±0·41
	8	10·5 ±0·29	—	9·6 ±0·24	9·1 ±0·16
	10	11·6 ±0·39	—	14·1* ±0·35	10·9 ±0·22
	13	12·3 ±0·51	—	13·6 ±0·48	12·9 ±0·52
Oil‡ (as a percentage of fresh wt.)	6	0·39 ±0·03	0·34 ±0·02	0·39 ±0·02	0·37 ±0·03
	8	0·54 ±0·03	0·54 ±0·01	0·63* ±0·04	0·51 ±0·04
	10	0·56 ±0·03	0·73* ±0·06	0·73* ±0·07	0·73* ±0·03
	13	0·71 ±0·08	0·81 ±0·01	0·77 ±0·06	0·61 ±0·03
Camphor‡ (percentage in oil)	6	70 ±1·3	73 ±2·0	73 ±2·1	70 ±2·0
	8	77 ±1·6	74 ±2·9	72* ±1·0	74 ±3·5
	10	66 ±2·1	55* ±2·7	68 ±3·0	68 ±1·6
	13	57 ±2·1	64* ±2·7	73* ±2·3	62 ±4·6

* Significantly different from control at 5% level. † Five replicates. ‡ Three replicates.

was gained by the controls when plants were harvested 5 weeks later. The survival of plants was 15–20% higher as a result of irradiation. The field trial thus conclusively

TABLE 4

GROWTH AND SURVIVAL OF *O. KILIMANDSCHARICUM* PLANTS FROM UNIRRADIATED AND IRRADIATED SEEDS

Values are means ± standard error

Age (weeks)	Parameter	Value at radiation dose of: 0	1500 R	2500 R
9	Leaves			
	Fresh weight (g)	2·8 ± 0·25	5·3* ± 0·89	5·9** ± 1·03
	Dry weight (mg)	290 ±22	642*** ± 81	724*** ±111
	Stem			
	Fresh weight (mg)	567 ±76	1511*** ±245	1753*** ±326
	Dry weight (mg)	136 ±14	257*** ± 32	291* ± 54
14	Fresh weight of leaves (g)	123 ±17	144 ± 21	130 ± 15
	Fresh weight of stem (g)	194 ±23	227 ± 37	226 ± 27
	Survival (%)	69 ± 4·0	82* ± 2·9	79† ± 3·4

*, **, *** Significantly different from controls at 5, 1, and <1% levels.
† Significantly different from controls at 10% level.

confirmed the significantly positive, yet transient, response of *O. kilimandscharicum* to X-irradiation of its seeds.

IV. Discussion

One of the observations of this investigation is related to the earlier and greater amount of germination induced by radiation at 500 and 1500 R but not at 2500 R. Similar results have been reported in wheat, barley, and maize by Suss (1966) and by Coutinho (1971) in grape cv. Fernao Pires. Zelles *et al.* (1971) have observed that ultraviolet irradiation ($0 \cdot 024$ J cm^{-2}) of *Pinus sylvestris* pollen stimulated the actinomycin-sensitive (requiring newly synthesized *m*-RNA) but not the actinomycin-insensitive (controlled by long-lived *m*-RNA) phase of germination.

As far as the authors are aware, this is the first report on the stimulation of growth by irradiation of seeds of an essential oil plant, though similar effects, if not so marked, have been observed in other plant species. Suss (1966) has reported increases in height, fresh weight, and dry weight of plants after low-dose γ-irradiation of barley, wheat, and maize seeds. The increase in dry matter may be due to stimulation by radiation of primary physiological processes such as photosynthesis and nutrient uptake (Zhezhel 1958; Vlasyuk *et al.* 1959). If chlorophyll content per unit fresh weight is taken as an index of photosynthesis, our results (Table 3) indicate that this basic process was not affected.

The fact that the leaf : stem ratio changed at various radiation doses shows that leaves responded to radiation more than stems. A difference in the radiation response of different organs has been reported by Johnstone and Klepinger (1967). On γ-irradiation of *Yucca brevifolia* seeds with growth-inhibitory doses, they found roots to be more radiosensitive than shoots. An almost identical leaf : stem ratio for control and experimental plants in advanced growth stages shows that the stimulatory effects tended to disappear with passage of time. The transient nature of radiation stimulation is specially clear from Table 2. A better start for radiation-treated plants at transplantation time could lead to greater survival. This would give an added advantage to the radiation-induced stimulation and thereby lead to an overall increase in yield.

Even though there was no consistent trend in radiation effect on oil and camphor contents, the final yield of these constituents did increase because of the marked leaf stimulation. Similarly Stan and Croitoru (1971) could not find any effect of low-dose γ-irradiation, up to 10 kR on oil and protein contents in green leaves of soybean, although an increase in the net yield followed the vegetative stimulation.

Early flowering, as observed here, has been also reported by Sparrow (1966) in plants raised from *Brassica pebinensis* seeds irradiated with 20–60 kR X-rays. It is noteworthy that there was less stimulation in flowering at 500 R compared with lower and higher doses. Such an effect was also observed in the present study for stem dry weight (Table 2), though to a lesser extent. The effect on flowering was quite marked from the 10th to the 13th week. This wave-shaped dose–response curve was reported by Suss (1966) for different cereal varieties. Such a pattern could perhaps be explained in terms of differential sensitivity of various plant processes and their relative contribution to the property studied.

If germination, growth, and development (flowering) are considered as various stages of a continuous process dependent on endogenous hormonal balance, one may attribute the low-dose radiation stimulation to a favourable disturbance in this balance. Growth-inhibitory radiation doses are already known to lower endogenous indoleacetic acid (Gordon 1957; Deshmukh *et al.* 1965; Revin *et al.* 1970) and gibberellic acid (Sideris *et al.* 1971) and increase ethylene (Ogawa and Uritani 1970) levels. Such information is not yet available for low-dose effects.

It may be concluded that low-dose irradiation of seeds of *O. kilimandscharicum* has induced marked stimulation in vegetative growth, resulting in higher total essential oil content per plant.

V. Acknowledgment

We acknowledge the help rendered by Mr. A. S. Nirale in the large-scale field trial.

VI. References

Anon. (1966).—Int. atom. Energy Ag. Tech. Rep. Ser. No. 64.

Breslavets, L. P. (1946).—"Plants and X-rays." p. 192. (Academy of Science Press: Moscow.)

Bruisma, J. (1963).—The quantitative analysis of chlorophyll 'a' and 'b' in plant extracts. *Phytochem. & Phytobiol.* **2**, 241–7.

Coutinho, M. P. (1971).—Preliminary results of X-ray applications on grape. *Stimulation Newsl.* **2**, 1–4.

Deshmukh, A. D., Joshi, R. K., and Gaur, B. K. (1965).—Reduction of endogenous hormone level in plant by ionizing radiation. Proc. Semin. Int. Cell Biol., Bombay, pp. 260–5.

Ehrenberg, L. (1971).—Isotopes and radiations in plant amelioration. *Agrochimica* **15**, 138–49.

Gordon, S. A. (1957).—The effect of ionizing radiation on plants: biochemical and physiological aspects. *Q. Rev. Biol.* **32**, 3–14.

Johnson, E. L. (1936).—Susceptibility of seventy species of flowering plants to X-irradiation. *Pl. Physiol., Lancaster* **11**, 319–42.

Johnstone, G. R., and Klepinger, F. W. (1967).—The effect of gamma radiation on germination and seedling development of *Yucca brevifolia* Englen. *Radiat. Bot.* **7**, 385–8.

Kuzin, A. M. (1956).—The utilization of ionizing radiation in agriculture. Proc. 1st Int. Conf. Peaceful Uses of Atomic Energy, Geneva, 1955. Vol. 12. pp. 149–58.

Ogawa, M., and Uritani, I. (1970).—Effect of gamma radiation on peroxidase development in sweet potato discs. *Radiat. Res.* **41**, 342–51.

Revin, A. F., Vasiloi, Yu. V., and Narimanov, A. S. (1970).—Effect of gamma irradiation on trytophan synthetase activity in plant seedlings. *Soviet Pl. Physiol.* **17**, 1064–5.

Sax, K. (1955).—The effect of ionizing radiation on plant growth. *Am. J. Bot.* **41**, 360–4.

Sax, K. (1963).—The stimulation of plant growth by ionizing radiation. *Radiat. Bot.* **3**, 179–86.

Sax, K., and Schairer, L. A. (1963).—The effect of chronic gamma irradiation on apical dominance of trees. *Radiat. Bot.* **3**, 283–5.

Sideris, E. G., Nawar, M. M., and Nilan, R. A. (1971).—Effect of gamma radiation on GA solution and gibberellin-like substances in barley seedlings. *Radiat. Bot.* **11**, 209–14.

Sparrow, A. H. (1966).—Plant growth stimulation by ionizing radiation. Int. atom. Energy Ag. Tech. Rep. Ser. No. 64. pp. 12–15.

Stan, S., and Croitoru, A. (1971).—Effects of low, moderate and high levels of gamma radiations (^{60}Co) on soybean plants. II. Changes in protein, oil and micronutrient content. *Stimulation Newsl.* **2**, 11–4.

Suss, A. (1966).—Effect of low doses of seed irradiation on plant growth. Int. atom. Energy Ag. Tech. Rep. Ser. No. 64. pp. 1–11.

Vlasyuk, P. A., Pochinok, Kh. N., and Grodzinskii, D. M. (1959).—The influence of nuclear radiations on the intensity of photosynthesis. *Trudy ukr. nauchno-issled. Inst. Fiziol. Rast.* **16**, 77–82.

Zelles, L., Ernest, D. E. W., and Tiffe, H. W. (1971).—Stimulation of the germination of *Pinus sylvestris* pollen by UV-radiation. *Stimulation Newsl.* **2**, 54–6.

Zhezhel, N. G. (1958).—Studies on the mechanism of the biological effect of small doses of ionizing radiation in plants. *Vest. sel.-khoz. Nauki, Mosk.* **8**, 123–6. [*Biol. Abstr.* **45**, 88387 (1964).]