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Effect of γ - irradiation on seedling growth and endogenous level of IAA in seedlings and callus of *Punica granatum* L. cv. Ganesh

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ABSTRACT

Purpose of the present work was to study whether the effect of γ- radiation doses given to dry an soaked (where germination initiation has begun) seeds of Punica granatum, are carried to the explants also that are used for in vitro culture or is it limited to the seedling growth only. To investigate the effect of γ- radiation on endogenous level and radiolysis of IAA; soaked and dry seeds were irradiated with 1, 5, 10 and 15 kR of γ- radiation and germinated in vitro. Callus was regenerated from various explants of these seedlings. Endogenous level of IAA in root, stem and callus and radiolysis of IAA in different media viz. aqueous or cell extract was recorded. All doses, except 1 kR, inhibited the seedling growth. Callus initiation and growth was delayed by 10 and 15 kR, whereas 1 and 5 kR stimulated the callus growth. Endogenous level of IAA in root, stem and callus was suppressed by all the doses of γ- radiation, except 1 kR; where it was more. Radiolysis of IAA in aqueous and cell extract was less than radiolysis of IAA crystasl. Higher doses of γ inhibited growth of seedlings, callus derived from them and endogenous level of IAA. As compared to pure IAA, IAA present in aqueous solution or in cell extract had less radiolysis.

Key Words: γ-irradiation, Radiolysis, IAA, *Punica granatum*,

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INTRODUCTION

γ-irradiation is known to significantly affect Physiological and Biochemical processes in plants e.g. it disturbs the synthesis of protein [1], hormone balance [2], leaf gas-exchange [3 , water exchange and enzyme activity [4]. Effect of λ radiations on peroxidase and protease activity, lipid peroxidation, protein content, seed germination and seedling growth in chick pea have also been demonstrated [5].

Depending on the doses of γ – irradiation given to the seeds, both stimulatory as well as inhibitory effects on seedling growth [6, 7] have been reported. It has been observed that soaked

seeds of *Punica granatum*, when irradiated with 1 kR of γ – rays had better root growth over the control, while all the doses above 1 kR were found to be inhibitory to shoot and root growth [8].

Since both seedling as well as root growth are very much dependent on mitotic activity and auxin content, therefore, analysis of mitotic index and endogenous level of IAA in the seedlings germinated from the irradiated seeds was thought desirable for the present work.

Moreover, attempts were made to use the seedlings derived from irradiated seeds as source of various explants and their callusing capacity. Whether the callus derived from irradiated seedlings explants had any alteration in IAA content was also studied.

MATERIALS AND METHODS

γ- Irradiation of Seeds: Dry seeds (DIS) as well as seeds soaked (SIS) for 24 h of *Punica granatum* L var. Ganesh were irradiated with 1, 5, 10 and 15 kR of γ – rays from ^{60}Co source (IIT Bombay), which emits 200 megarads/hr. Prior to use all seeds were surface sterilized with 0.1% $HgCl_2$.

Seed Germination: Hundred irradiated seeds of each dose and control were germinated aseptically on half strength MS [9] medium at 25^{0}C in dark for one week and then transferred to 16 hrs light and 8 hrs dark periods. Light intensity was maintained at 3000 Lux. On 35th day percentage of germination, root and stem length, their fresh and dry weight, number of leaves and root laterals of germinated seedlings were recorded.

Callus generation form different explants: From 35 days old seedlings of γ-irradiated as well control; approximately 0.5 cm. segments of root, hypocotyl, cotyledonary node, cotyledon, internode, node, leaf and shoot tip were cut and inoculated onto B5 medium [10], augmented with 0.5 mg l^{-1} 2,4 – D, 3% sucrose and 0.8% agar. Callus was allowed to grow for 6 weeks. Seedlings from 15 kR irradiated soaked seeds did not have completely opened leaves; therefore, the outer most leaf primordia were taken.

IAA Estimation*:* IAA content in root, shoot and cotyledon of 21 days old seedlings as well as six weeks old callus derived from 8 different explants, was analyzed by Tang and Bonner's method [11]. IAA was extracted from tissues in chilled 80% methanol. Cell debris from the methanol extract was removed by centrifugation at 4^0 C. Clear extract was treated with Salkowski reagent, color was allowed to develop for 30 minutes and optical density was measured at 535 nm

Radiolysis of IAA by γ-radiation:

(i) ***IAA crystals:*** 5 mg of IAA crystals was irradiated with 1,5,10 and 15 kR of -radiation and then dissolved in 100 ml double distilled water. The λ max was recorded between 200 – 300 nm and was compared with non-irradiated IAA solution of the same concentration.

(ii) ***IAA aqueous solution***: In another set of experiment 5 mg of IAA was dissolved in HPLC grade Mili Q water and then irradiated with above mentioned doses of γ –rays. Their λ max was recorded at 200 – 300 nm.

(iii) ***IAA with cell extract solution:*** 500 mg of seedling tissues (either root or shoot) were macerated on ice and cell content was extracted in chilled PO_4 buffer (pH 7) then centrifuged at 0^0 C at 8000 rpm and supernatant was taken as cell extract. Similarly IAA was extracted from

callus also. To this extract 2 ml (of 1mg/ml) IAA solution was added, it was made up to 20 ml with PO_4 buffer and then irradiated with 1, 5, 10 and 15 kR of γ-rays. The λ max was recorded at 200 – 300 nm and compared with control.

RESULTS

Seed Germination: Emergence of radical (taken as initiation of germination) from the 1 and 5 kR SIS and DIS were same as control i.e. 48 hours; whereas both 10 and 15 kR SIS and DIS caused a delay of 24 hours and seeds germinated after 72 hours (Table I). 100% germination was observed in control as well as all the treated seeds.

Figure 1 Effect of various doses of γ-rays, given to (A) Soaked and (B) Dry seeds on growth of seedlings, as recorded on 35th day after treating and sowing in vitro

A

B

Table – I. Effect of various doses of γ - rays given to soaked seeds (SIS) and Dry seeds (DIS) of *Punica granatum* on germination and seedling growth
(Results are mean of 100 values ± S.E.)

γ - dose	Germination Initiation		Germination Percentage (%)		Seedling growth on 35th day											
					SIS						DIS					
	SIS (Hr)	DIS (Hr)	SIS	DIS	Root length (cm)	Shoot Length (cm)	No. Of Leaves	No. of Root laterals	Fresh Wt. of Seedling (g)	Dry Wt. of Seedling (g)	Root length (cm)	Shoot Length (cm)	No. Of Leaves	No. of Root laterals	Fresh Wt. of Seedling (g)	Dry Wt. of Seedling (g)
Control	48	48	100	100	5.30 ±0.08	5.48 ±0.03	6.0 ±02	15 ±0.07	155.0 ±0.05	15.45 ±0.08	5.30 ±0.08	5.48 ±0.03	6.0 ± 02	15 ± 0.07	155.0 ±0.05	15.45 ±0.08
1 kR	48	48	100	100	**5.60** ±0.04	4.80 ±0.05	6.0 ±08	**18** ±0.01	118.0 ±0.02	11.65 ±0.09	5.04 ±0.05	4.31 ±0.08	6.0 ± 08	12 ± 0.07	151.0 ±0.05	15.0 ±0.08
5 kR	48	48	100	100	4.11 ±0.06	2.55 ±0.02	6.0 ±05	22 ±0.04	93.0 ±0.02	9.10 ±0.09	4.48 ±0.03	3.32 ±0.06	6.0 ± 06	13 ± 0.07	105.0 ±0.04	10.4 ±0.03
10 kR	72	72	100	100	3.02 ±0.05	1.41 ±0.04	4.0 ±09	15 ±0.06	88.0 ±0.04	8.55 ±0.07	4.25 ±0.07	2.56 ±0.01	6.0 ± 04	12 ± 0.09	93.0 ±0.08	9.13 ±0.02
15 kR	72	72	100	100	1.98 ±0.03	0.91 ±0.09	0	5 ±0.05	80.0 ±0.01	7.75 ±0.02	4.15 ±0.09	2.40. ±0.09	0	12 ± 0.04	86.0 ± 0.07	8.40 ±0.04

Table – II. Fresh & Dry weight of callus (as recorded on 60th day after inoculation) initiated from different explants from seedlings derived from Soaked and γ-irradiated seeds (SIS) and Dry irradiated seeds (DIS) of *Punica granatum* L Var. Ganesh. Initial inoculated fresh weight of callus was 50 mg (Values are mean ± S.E. of 10 samples)

(C = Control, FW = fresh weight; DW = dry weight, DM = % dry mass, Coty = Cotyledon)

γ- ray dose kR	Root Callus			Hypocotyl Callus			Coty. nodes Callus			Coty Leaf Callus			Inter-Node Callus			Node Callus			Leaf Callus			Shoot Tip Callus		
	FW	DW	DM	FW	DW	DM	FW	DW	DM	FW	DW	DM	FW	DW	DM	FW	DW	DM	FW	DW	DM	FW	DW	DM
SIS																								
Control	250 ± 2.0	20.5 ± 0.08	8.2	262 ± 5.8	21.2 ± 0.05	8.09	214 ± 6.8	17.1 ± 0.02	7.99	210 ± 9.5	16.8 ± 0.02	8.0	374 ± 9.7	30.0 ± 0.4	8.02	212 ± 5.7	17.0 ± 0.07	8.01	175 ± 5.1	14.1 ± 0.06	8.05	282 ± 5.8	22.6 ± 0.05	8.01
1	**270± 3.0**	21.6 ± 0.03	8.0	**313 ± 4.8**	25.5 ± 0.07	8.14	**232 ± 17**	18.5 ± 0.09	7.97	**315 ± 10.5**	25.7 ± 0.07	8.15	**419 ± 8.7**	33.5 ± 0.09	7.99	**235 ± 2.9**	18.8 ± 0.08	8.0	**206 ± 9.1**	16.4 ± 0.09	7.96	**289 ± 9.7**	22.5 ± 0.07	7.99
5	**261± 2.8**	20.9 ± 0.01	8.0	**332 ± 11**	26.5 ± 0.09	7.98	**250 ± 13**	20.1± 0.01	8.04	**350 ± 10.8**	28.1 ± 0.06	8.02	**493 ± 6.7**	39.5 ± 0.02	8.01	**266 ± 10.8**	21.3 ± 0.06	8.0	**214 ± 6.7**	17.3 ± 0.07	8.08	**344 ± 10.5**	27.5 ± 0.06	7.98
10	227± 9.8	18.1 ± 0.09	8.2	209 ± 15.4	16.8 ± 0.01	8.03	182 ± 17	14.6 ± 0.08	8.02	176 ± 2.9	14.1 ± 0.03	8.01	294 ± 10.8	23.6 ± 0.05	8.02	198 ± 12.7	15.9 ± 0.07	8.03	**185 ± 7.9**	14.8 ± 0.01	8.0	223 ± 3.8	17.8 ± 0.02	7.99
15	220± 2.7	18.0 ± 0.04	8.2	189 ± 14.2	15.0 ± 0.06	7.93	171 ± 11	13.8 ± 0.05	8.07	162 ± 10.9	13.0 ± 0.07	8.02	--	--	--	--	--		--	--		214 ± 9.2	17.1 ± 0.03	
DIS																								
Control	FW	DW	DM	FW	DW	DM	FW	DW	DM	FW	DW	DM	FW	DW	DM	FW	DW	DM	FW	DW	DM	FW	DW	DM
1	**254± 11**	20.3 ± 0.08	7.9	**295 ± 2.8**	24.0 ± 0.07	8.13	**283 ± 13**	23.0 ± 0.06	8.12	**387 ± 6.8**	31.0 ± 0.09	8.01	**389 ± 8.5**	31.2 ± 0.08	8.02	**299 ± 6.8**	23.2 ± 0.07	8.0	**185± 12.0**	15.0 ± 0.05	8.10	**300 ± 10.0**	24.0 ± 0.05	8.0
5	**252± 10**	20.1 ± 0.08	7,9	**271 ± 13.5**	21.6 ± 0.06	7.97	**271 ± 11**	22.0 ± 0.04	8.11	**340 ± 10.8**	27.0 ± 0.04	7.94	**462 ± 2.7**	40.0 ± 0.05	8.00	**339 ± 6.2**	27.2 ± 0.08	8.02	**228 ± 8.7**	18.3 ± 0.06	7.99	**331 ± 14.5**	26.6 ± 0.02	8.3
10	232± 2.4	18.7 ± 0.04	8.1	246 ± 12.4	19.6 ± 0.03	7.96	198 ± 11	15.9 ± 0.07	8.03	285 ± 6.7	23.0 ± 0.01	8.07	320 ± 2.9	25.7 ± 0.07	8.03	179 ± 9.7	14.3 ± 0.01	7.98	162 ± 9.7	13.0 ± 0.01	8.02	193 ± 3.7	15.5 ± 0.07	8.3
15	175± 1.9	13.5 ± 0.05	7.7	210 ± 13.2	16.8 ± 0.09	8.0	150 ± 10	12.1 ± 0.03	8.06	276 ± 9.7	22.0 ± 0.03	7.97	281 ± .8	22.6 ± 0.02	8.04	147 ± 10.9	11.8 ± o.06	8.02	130 ± 10.5	10.4 ± 0.09	8.0	182 ± 1.8	14.6 ± 0.01	8.7

Seedling Growth: Data presented in Table 1 shows that none of the radiation doses could enhance the growth of aerial parts viz. shoot, leaf etc. However, an increase in the length of main root and number of lateral roots was noted in seedlings from SIS 1 kR irradiated seeds. With increased γ – radiation doses there was a decrease in root, shoot and leaf growth, even the size of cotyledonary leaves was slightly reduced (Figure 1 and Table I). In seedlings from SIS with 15 kR, leaves did not open up till 21st days and remained as part of shoot apex.

Callusing*:* Callus initiation occurred from all the explants. However, the time taken for callus initiation varied in different explants and the γ – radiation doses given to the seeds prior to germination (Table II). All the explants from control, 1 and 5 kR irradiated seeds showed callus initiation by 7th day, and 10 and 15 kR on 8th and 10th days respectively.

Fresh weight of callus (as measured on 60th day) derived from all the SIS irradiated explants, showed increase at 1 and 5 kR. It was more in 5 kR treated ones. 10 and 15 kR inhibited the fresh weight of the callus. However, ratio of fresh to dry weight i.e. % dry mass remained almost unaffected i.e. in the range of 7.93% to 8.2%. Almost similar results were obtained from DIS irradiated set of experiments.

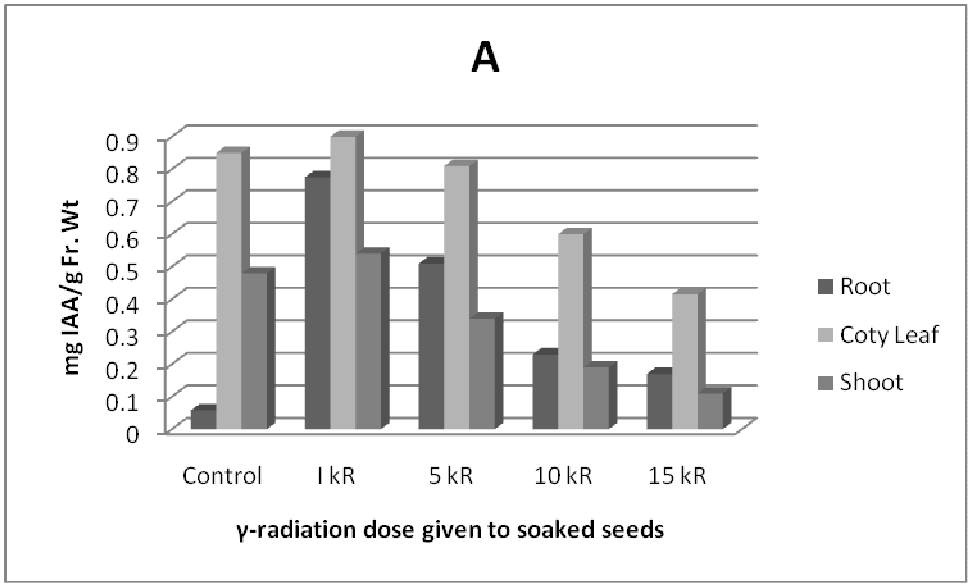
Indole Acetic Acid (IAA) Content in Seedling Tissues: IAA content was analyzed in shoot, root, and cotyledonary leaf of the seedlings from SIS and DIS.

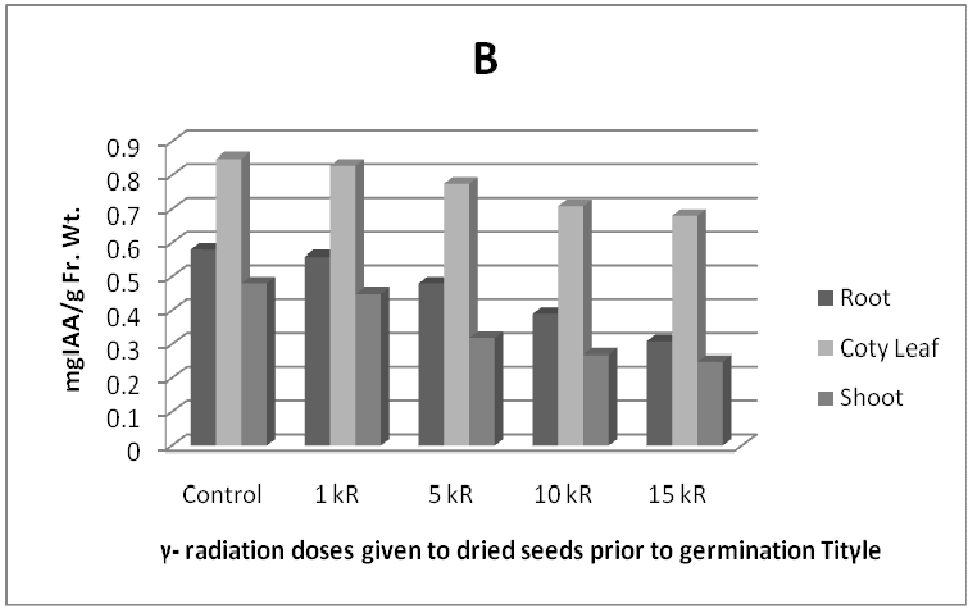
Root - There was significant increase in IAA content of root, taken from SIS seedlings at 1 kR. Rest all the doses inhibited IAA contents in the roots. But DIS root showed inhibition in IAA content at all the treatments.

Cotyledonary Leaf and Shoot tip – Cotyledonary leaf *had* higher amount of IAA than root and shoot tip. But the impact of γ-doses was the same as on roots in both SIS and DIS of cotyledonary leaf and shoot tip.

Increase in IAA content in 1kR irradiated SIS was 30% for root 12% for shoot tip and only about 6% for cotyledonary leaf which had highest amount of endogenous level of IAA at 10 and 15 kR shoots, roots and cotyledonary leaves from (Figure 2) SIS showed much more inhibition than from DIS treated ones. Control shoots had 0.85 mg g^{-1} and lowest in shoot was 0.48 mg g^{-1}; whereas root had 0.58 mg g^{-1} IAA.

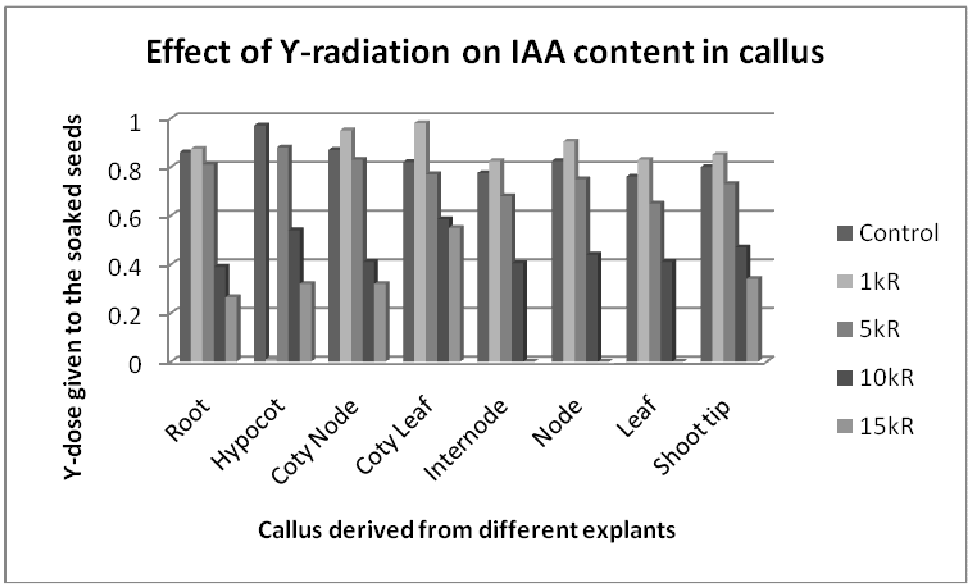
Figure 2. Effect of various doses of γ-radiation given to (A) Soaked and (B) Dry seeds of *Punica granatum var. Ganesh*; on the endogenous level of IAA in 3 different organs of 35 days old seedlings (Resulta are mean of 10 values).

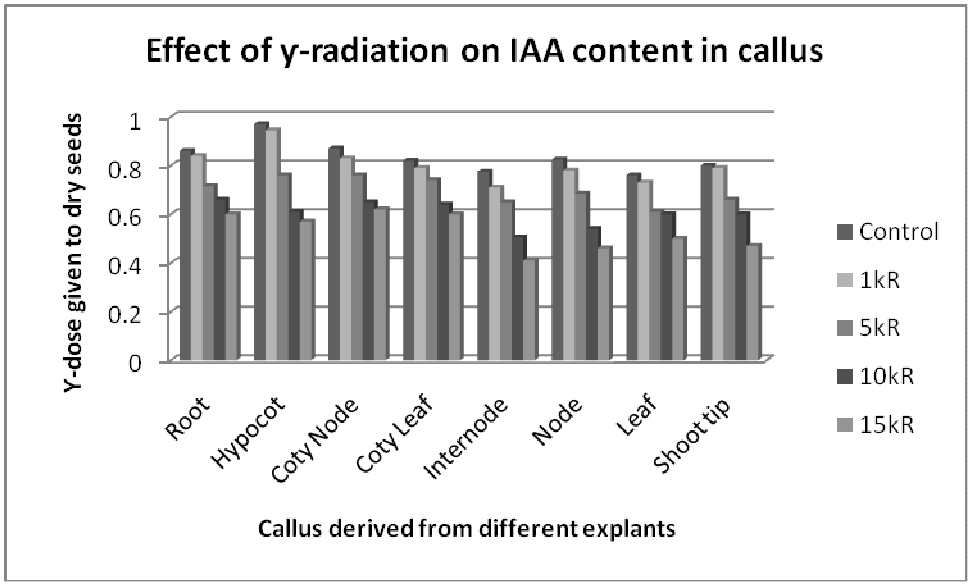




Indole Acetic Acid (IAA) Content in Callus: When IAA content was assayed in callus derived from 8 different explants (root, hypocotyl, cotyledonary node, cotyledonary leaf, internode, node, leaf, shoot tip) from seedling grown from γ – irradiated and control seeds (Figure 3), maximum IAA was found in callus derived from hypocotyl (0.97 mg g^{-1}). It was interesting to note that IAA content of callus derived from various explants grown from γ - irradiated dry or soaked seeds showed exactly similar response to different doses (1, 5, 10 and 15 kR) of γ – irradiation as shown by seedling tissues; i.e. only 1 kR increased the endogenous level of IAA in explants derived from SIS seedlings. Whereas DIS showed more inhibition at 1 and 5 kR doses of γ – rays.

Figure 3. Effect of various doses of γ-radiation given to Soaked & Dried seeds of *Punica granatum var. Ganesh*; on the endogenous level of IAA in callus derived from 8 different explants of 35 days old seedlings (Results are mean of 10 values).





Radiolysis of IAA Crystals: Radiolysis of IAA due to γ-rays significantly increased with increase in doses of γ-rays. As compared to control (non-irradiated IAA) there was 14%, 26%, 30% and 34% radiolysis of IAA at 1, 5, 10 & 15 kR doses. λ max scanning of irradiated IAA crystals between 200 – 300 nm did not show any isobestic point.

Radiolysis of IAA aqueous solution: IAA in solution also showed increased radiolysis with increase in doses of γ-rays. However, radiolysis of IAA by all doses was approximately 5 times less as compared to radiolysis of IAA crystals.

Radiolysis of IAA dissolved in plant extract: Table – 3 shows that there was less radiolysis of IAA when root extract was added as compared to shoot extract.

Table – III. Radiolysis of IAA crystals, IAA in aqueous solution and IAA in presence of cell extracts from root and shoot.

γ- dose in kR	IAA Crystal		IAA Aq. Solution		IAA + Root extract		IAA + Shoot Extract	
	µg/ml	Radiolysis	µg/ml	Radiolysis	µg/ml	Radiolysis	µg/ml	Radiolysis
0	50	0%	50	0%	100	0%	100	0%
1	43.3 ± 0.03	13.4%	48.4 ± 0.05	3.2%	99.4 ± 0.06	0.6%	99.2 ±0.08	0.8%
5	37.1 ±0.03	25.8%	47.7 ±0.07	4.6%	99.4 ± 0.02	0.8%	98.2 ±0.02	1.8%
10	35.3 ±0.07	29.4%	47.0 ±0.02	6.0%	98.94 ± 0.04	1.1%	99.0 ±0.04	2.0%
15	33.6 ±0.09	32.8%	46.5 ±0.05	7.0%	96.9 ±0.08	2.6%	97.4 ±0.09	3.1%

Table – IV. Mitotic index of records made from the root apex meristem of seedlings Derived from DIS & SIS seeds

γ- dose given to *P. granatum* seeds	Mitotic Index of Root Meristem of Seedlings Derived from	
	SIS	DIS
0 kR	**2.0**	**2.0**
1 kR	**2.5**	**2.2**
5 kR	**3.0**	**2.5**
10 kR	**3.6**	**2.8**
15 kR	**7.0**	**3.5**

DISCUSSION

Germination and Seedling growth: Though a dose dependent radiation induced seedling injury in 8 days old seedlings from irradiated barley seeds have been reported. From the seed germination data (Table I), it can be assumed that doses tried in the present work were apparently not damaging the embryo. Stimulatory effect of 1 and 5 kR of γ – radiation on general seedlings growth of *Pisum sativum* cv. dwarf green and *Vicia faba* respectively have been reported [7, 12]. But doses above 5 kR were inhibitory. Whereas in the *Punica granatum* we have found that increase in seedling growth stimulation was noted only in seedlings derived from SIS with 1 kR. All the other doses given to SIS and all doses given to DIS were inhibitory. Inhibitory effect of 1 kR on the seedling growth of *Sorghum vulgare* have also been reported [13], suggesting that effect of γ – rays on seedlings growth varies from species to species.

In a study of the effect of γ- irradiation on the subsequent germination and growth of irradiated seeds of maize, okra and groundnut it was found that the number of germinated seeds and the growth rate for the crops decrease with increase in the radiation dose the seeds were exposed to [14]. Inhibitory effects of higher doses of radiation on the shoot and root lengths of germinated seedling of irradiated seeds of *Cicer arietinum* Ladiz, *C. reticulatum* Ladiz. and *C. bijugum* K.H. Rech have also been observed [15]. Recently γ- radiation effect on growth of *Lepidium sativum* L. have been recorded, which showed that when the seeds are irradiated with 20 – 80 kR of γ-radiation ; though the seed germination was not much affected but the growth of all parts of seedlings showed declining tendency with increasing γ- radiations [16]. They concluded that Biochemical parameters like protein contents, protease, peroxidase and lipid peroxidation may be helpful in early assessment of effectiveness and superiority of irradiation dose.

Callussing: There have been reports of irradiation of cultured plant tissues and callus [17, 18]. However, we have not come across any paper where seedlings from irradiated seeds were taken as the source of the explants to generate callus. Our intention was to see whether irradiation doses given to seed (Soaked or Dry) prior to germination cause any variation that can affect the

in vitro growth of callus. We can deduce that inhibitory growth effect (by γ-radiation) of seedling is carried over during *in vitro* culture also. Hence the callus growth was inhibited at higher concentration

IAA Content: Although IAA content increased in all the 3 tissues (root, shoot and cotyledonary leaf) of the SIS treated seedling at 1 kR, but it stimulated the growth of roots only (Table 1). Thus suggesting that increased IAA level might have been directed towards growth of root and lateral roots. The results mentioned above suggested that IAA present in soaked seeds (where initial germination process had begun) must have got radiolyzed at higher doses (10 and 15 kR) of γ – rays. Radiolysis increases with increasing doses of γ - radiation.

Therefore, radiolysis of IAA to which crude extracts of root and shoot was added was also recorded.

Radiolysis of IAA: The results of IAA content in root shoot and hypocotyl clearly shows an inhibition in endogenous level of IAA when treated with higher doses of γ-radiation. To see whether it was due to radiolysis of IAA present in the tissue or not; a set of experiments were performed where IAA crystals, IAA solution and IAA along with root and shoot extract were γ-irradiated and their λ max was recorded. Radiolysis was compared on the basis of IAA present in the solution.
Maximum radiolysis was observed when IAA crystals were irradiated.

Aqueous solution of IAA showed nearly 5 times less radiolysis than IAA crystals. Radiolysis of IAA solution treated with such low doses of γ-rays might have broken only the carboxyl and methyl group of IAA which could have joined back in aqueous solution, hence less radiolysis was observed.

Radiolysis was more pronounced when shoot extract was added to IAA than the root extract. In young seedlings, roots have more inherent IAA, moreover, it was noted that (Table 1) irradiated roots had increased level of IAA.

It has been shown [19] that IAA in aqueous solution gets radiolyzed into Indole derivatives with some of them having properties similar to IAA; and the U.V. spectra of irradiated IAA solution showed 3 isobestic points and absorption at 245 nm.

In present study, when IAA solution was irradiated, only 2 isobestic points were seen, whereas when IAA was extracted from seed irradiated roots no isobestic point was noted and from irradiated seeds derived shoot only one isobestic point was seen. These results obviously showed differential effect of γ – rays on IAA present in a solution and present in the tissues. A detailed analysis of radiolysis of IAA by γ – irradiation is being carried out.

CONCLUSION

In can be concluded that γ-radiation given to seeds though did not damage the embryos, as they allowed 100% germination, but they did have inhibitory effect on the seedling growth, which increased with increase in doses. However, root growth was less inhibited which could be contributed to mitotic index. Mitosis was more inhibited in seedlings derived from SIS than DIS. Radiolysis of IAA study showed that as compared to crystals of IAA, IAA in aqueous solution and in cell extract showed less radiolysis.

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