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γ- Irradiation of seeds of *Punica granatum* L. cv. Ganesh inhibits amylase activity and seedling growth

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ABSTRACT

Effect of γ- irradiation (1, 5, 10 and 15 kR) on soaked and dry seeds of Punica granatum; on the in vitro germination, seedling growth, mitotic index and amylase activity were studied. Germination initiation was 100% in all the treated and control seeds, however time taken to initiate the germination varied. Control and seeds treated with 1 and 5 kR germinated after 48 hrs, whereas those treated with 10 and 15 kR had delayed germination (i.e. after 72 hrs). Seedling growth was more inhibited when soaked seeds were irradiated than with dry seeds at all the doses of γ-irradiation. There was an arrest of mitotic index with increasing dose. With increasing dose of γ-irradiation, in both soaked and dry irradiated seeds, similar trend of decrease in amylase activity was noted i.e. in control and 1 kR treated seed amylase activity reached its peak on 7th day. With increase in irradiation dose, the time taken for maximum amylase activity decreased.

Keywords: γ- irradiation, Pomegranate, amylase, *Punica granatum.*

INTRODUCTION

Effect of γ-irradiation on amylase activity of seeds has been recorded in many plant species [1,2]. Moreover γ-rays have also been found to alter morphological features and physiological processes resulting in altered growth and development. There have been reports of increased yield in many varieties when treated with lower doses of γ-rays [3,4, 5, 6] . However, most of the work has been done on crops plants and comparatively fewer reports are available on effect of γ-irradiation on tropical fruits.

The present work is an attempt to study the effect of γ-irradiated seeds of pomegranate on early stages of seedling growth and amylase activity. Since amylase activity begins at the onset of

seed germination (i.e. when seeds start imbibing water), soaked as well as dry seeds were irradiated. A comparative account of response by soaked and dried seeds is presented here.

MATERIALS AND METHODS

Seeds of *Punica granatum* L. cv Ganesh were carefully drawn from mature ripe fruits. After removing the juice they were thoroughly washed under running tap water to remove the pulp remains on the seeds. Seeds were then dried in an oven at 40^0 C for 2 – 3 hrs.

Both dried as well as seeds soaked for 24 hrs were irradiated with γ-irradiation from ^{60}Co source. Radiation doses were 1, 5, 10 and 15 kR.

The irradiated seeds as well as control (non-irradiated) seeds were surface sterilized with 0.1% mercuric chloride solution for 3 min ad rinsed thoroughly with autoclaved distilled water. Seeds were further treated with 0.5% sodium hypochlorite solution with 2 drops of Tween – 20 / 100 ml for 5 min and rinsed thrice with autoclaved distilled water. Then the seed coat was aseptically removed and embryos with cotyledons were inoculated onto half strength MS [8] medium supplemented with 3% sucrose and 0.8% agar. Prior to autoclaving pH of the medium was adjusted to 5.7.

The parameters to study the effect of γ-irradiation on seedling growth were length of root and shoot, number of lateral roots and leaves of the seedlings at an interval of 7 days till 35th day. Mitotic index of seedlings from irradiated seeds was recorded on 7th day. Amylase activity of irradiated seeds was estimated after every 24 hrs upto 8th day after irradiation. The enzyme was extracted in phosphate buffer (pH 6.7). Soluble starch was taken as enzyme substrate. Enzyme activity was allowed to take place for 30 min and then was stopped by adding 0.5 ml 1N HCl. Amylase activity was assayed by the standard method [9] .

RESULTS AND DISCUSSION

Though 100% germination occurred in all the irradiated and control seeds, the time taken for seeds to germinate varied for different treatments. Control and seeds irradiated with 1 and 5 kR (whether dry or soaked) germinated within 48 hrs; whereas 10 and 15 kR treated seeds germinated after 72 hrs. It has been suggested that γ-rays delayed germination and even set in dormancy with increasing doses in *Vicia sativa* var. subplicota [10].

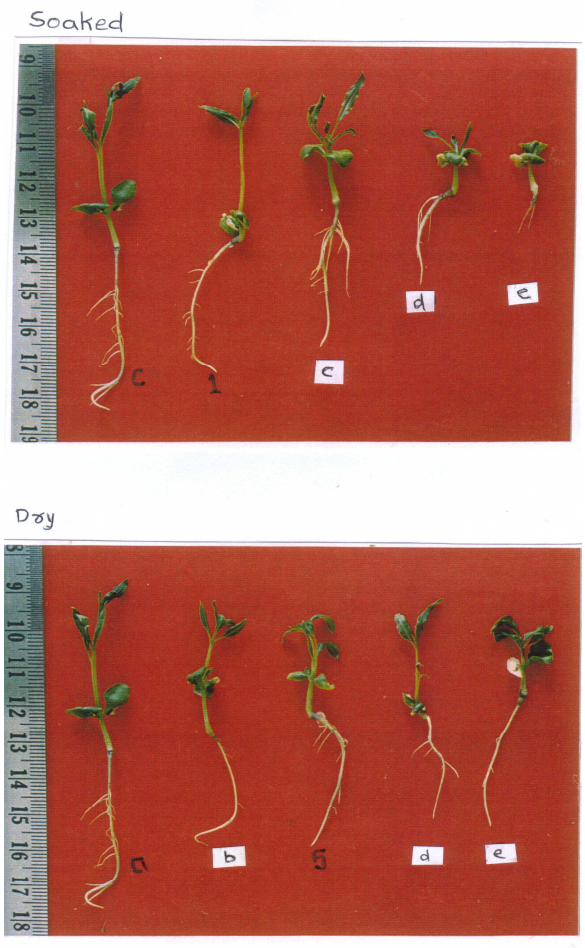
Table 1. Effect of various doses of γ-irradiation of soaked and dry seeds of *Punica granatum* L. cv. Ganesh on growth of *in vitro* grown seedlings on ½ strength MS medium as measured on 21st day after inoculation.

γ – doses in kR	Shoot length (cm) ± S.E.		Number of Leaves ± S.E.		Root length (cm) ± S.E.		No. Of lateral roots ± S.E.		Fresh weight of Seedling, (mg) ± S.E.		Dry weight of seedling (mg) ± S.E.	
	Soaked seeds	Dry seeds	Soaked seeds	Dry seeds	Soaked seeds	Dry seeds	Soaked seeds	Dry seeds	Soaked seeds	Dry seeds	Soaked seeds	Dry seeds
Control	2.32 ± 0.04	2.32 ± 0.04	4 ± 0.07	4 ± 0.07	3.83 ± 0.05	3.83 ± 0.05	10 ± 0.05	10 ± 0.05	60 ± 0.12	60 ± 0.12	12.00 ± 0.11	12.00 ± 0.11
1 kR	1.76 ± 0.04	1.01 ± 0.02	2 ± 0.03	4 ± 0.06	4.60 ± 0.09	3.83 ± 0.08	17 ± 0.07	8 ± 0.07	81 ± 0.15	79 ± 0.17	18.22 ± 0.09	17.38 ± 0.08
5 kR	1.21 ± 0.09	0.91 ± 0.04	2 ± 0.05	4 ± 0.04	3.80 ± 0.02	3.14 ± 0.06	13 ± 0.02	5 ± 0.02	70 ± 0.13	68 ± 0.15	14.84 ± 0.04	14.56 ± 0.05
10 kR	0.90 ± 0.01	0.81 ± 0.07	2 ± 0.03	2 ± 0.05	2.05 ± 0.04	3.00 ± 0.05	6 ± 0.06	4 ± 0.06	47 ± 0.09	56 ± 0.12	8.72 ± 0.09	10.70 ± 0.09
15 kR	0.97 ± 0.01	0.79 ± 0.06	Unopened leaf primordia	2 ± 0.02	1.02 ± 0.03	2.98 ± 0.03	4 ± 0.05	4 ± 0.05	40 ± 0.07	50 ± 0.09	6.91 ± 0.05	8.86 ± 0.07

Values are mean of 100 replicas of each reading.

All the tried doses of γ-irradiation were found to be inhibitory to seedling growth (figure 1 & Table 1) except the initiation and number of lateral roots. With increase in γ-irradiation dose the inhibition became more pronounced. As compared to root length, there was a greater decrease in shoot length in response to all the doses of γ-irradiation. Initiation of lateral roots started on 21st day in control, 10 and 15 kR treated seeds, whereas 1 kR hastened the lateral root primordia induction by one week. Moreover 1 and 5 kR γ-irradiation of soaked seeds stimulated number of lateral roots formed over control. All the irradiation doses given to dry seeds and 10 and 15 kR given to soaked seeds inhibited the growth of lateral roots.

Figure – 1. 21 days old seedlings of *Punica granatum* L cv Ganesh derived from Soaked γ-irradiated and Dry γ-irradiated seeds. (a = Control, b = 1kR, c= 5kR, d = 10 kR and e = 15kR γ-irradiated seeds)



First pair of leaves appeared in second week from control, 1 and 5 kR treated soaked seeds; third week in the seedlings from all the doses of γ-irradiated dry seeds and in fourth week from 10 and 15 kR irradiated soaked. Though initiation of leaf primordia slowed down with increased radiation doses; by 35th day all the doses given to dry irradiated seeds, and 1 and 5 kR to soaked seeds including control had 3 pairs of leaves Soaked irradiated seeds with 10 kR had only 2 pairs of leaves. No leaf initiation took place in seedling from 15 kR irradiated soaked seeds.

Stimulatory effect of 1 and 5 kR of γ-irradiation doses on seedling growth of *Pisum sativum* cv. Dwarf green and *Vicia faba* have been shown respectively by [4, 11]. Doses more than 5 kR were inhibitory; whereas 1 and 5 kR of γ-irradiation were found to be inhibitory to seedling growth of *Sorghum vulgare* [12]. In the present work none of the doses could stimulate seedling

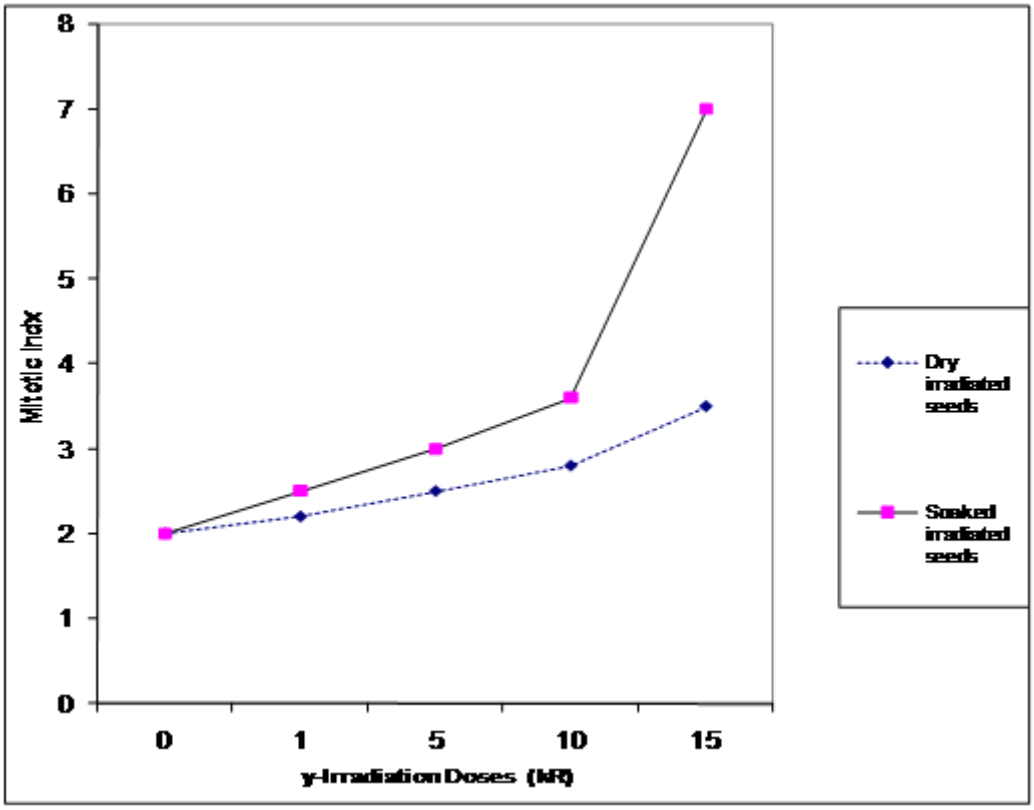
growth except stimulation in number of lateral roots (but not the growth of main root) by 1 and 5 kR doses given to soaked seeds. Thus suggesting that effect of γ-irradiation on seedling growth varied from species to species.

Mitotic index of seedlings which was recorded on 7th day after germination showed that with increase in radiation dose, the mitotic index increased, suggesting the arrest of mitosis. As seen in figure 2 there was a greater mitotic arrest when soaked seeds were irradiated with higher doses of γ-radiation (10 and 15 kR).

Decrease in mitosis and seedling vigor with increasing doses of γ-radiation in *Vigna radiata* L has been reported earlier [13]

Figure 2. Effect of various doses of γ-rays on Mitotic Index of seven days old seedlings grown from dry and soaked irradiated seeds of *Punica granatum* cv. Ganesh.

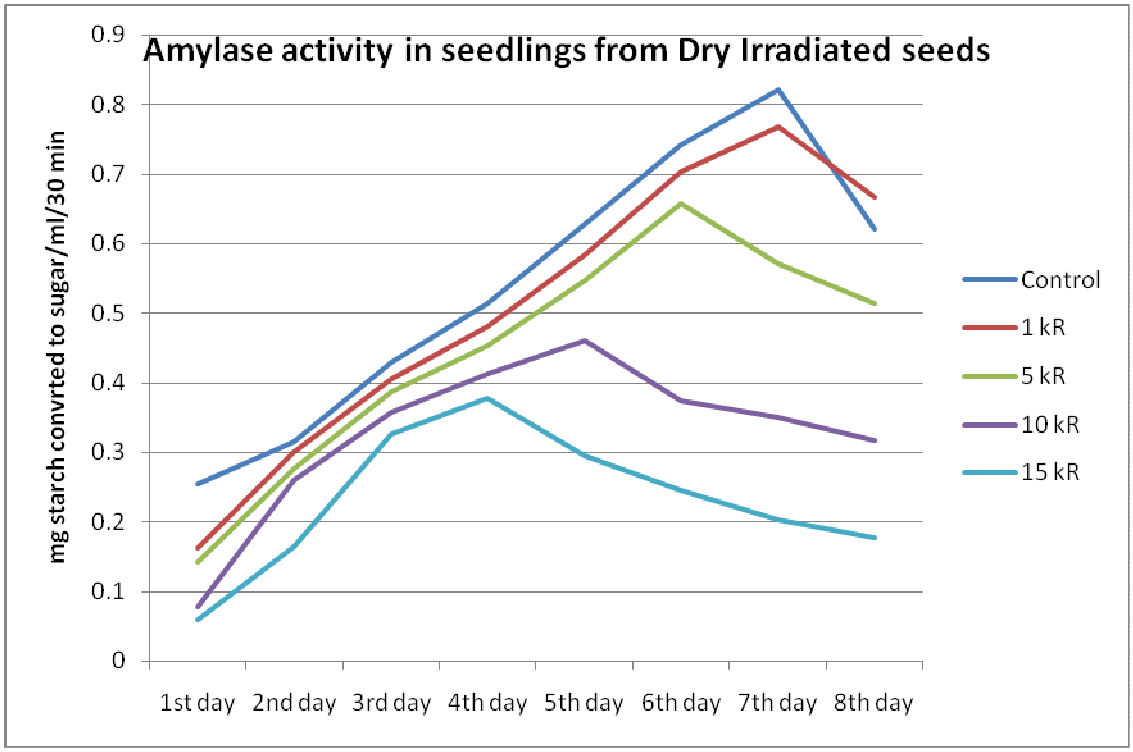
(—♦—Control —■— 1 kR, —Δ— 5 k R, —X— 1 0 k R, —Ж— 1 5 k R)

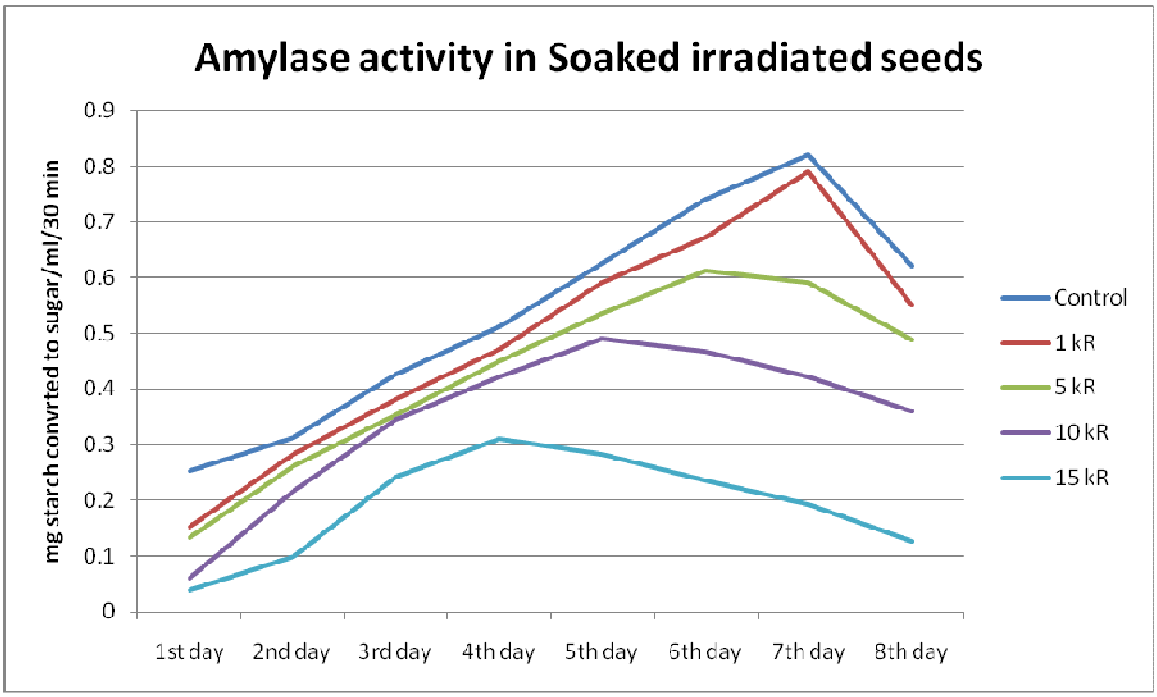


Germinating seedlings from seeds, which were soaked prior to γ-irradiation; exhibited more inhibition in seedling growth, at all the tried doses, as compared to dry-irradiated seeds. It is expected that soaked seeds must have started germination process by the time they were irradiated hence chances are that amylase activity might have been affected by γ-irradiation. Therefore it was thought desirable to study the amylase activity in seedlings from soaked and dry irradiated seeds.

Amylase activity measurement was started 24 hours after irradiation and after that at 24 hrs interval till amylase activity started to decrease.

Figure 3.Effect of various doses of γ-radiations given to SIS (soaked irradiated seeds) and DIS (dry irradiated seeds of *Punica granatum* on α-amylase activity as measured by conversion of starch into simple sugar/ml/30 min.





The amylase activity during initiation of seed germination for both dry and soaked seeds as well as control was in the range of 0.26 to 0.35 mg starch converted to sugar in 30 min. The time taken for this range of enzyme activity varied in seeds with different treatments. It was 48 hrs for control, 1 and 5 kR irradiated seeds and 72 hrs for 10 and 15 kR irradiated seeds (both soaked and dry). Thus showing a correlation between amylase activity and seed germination.

The amylase activity peaked on 7th day in control and 1 kR treated, 6th day in 5 kR treated, 5th day in 10 kR treated and 4th day in 15 kR treated both soaked and dry irradiated seeds (figure 3). Though trends of response by both soaked and dry treated seeds were the same, slight difference in amylase activity was noted in both the treatments. This could have been due to the fact that soaked irradiated seeds were soaked 24 hrs prior to irradiation hence the first reading after 24 hrs was actually after 48 hours of activating the enzyme. None of the tested doses were found to be stimulatory to amylase activity, though there have been reports of stimulatory effect of γ-radiation on amylase activity by 1 kR in maize [14].

After γ-irradiation, many workers noted change in amylase activity. While studying the effect of γ-irradiation on amylase activity in rice seedlings it was found that during germination it reached a peak on 8th day. Irradiation stimulated amylase activity at lower doses but inhibited at higher doses [16]. In cotyledon of *Cicer arietinum* var. L144 amylase activity increased upto 6th day and in another var. Hima upto 4th day [1]. An increase in radiation dose decreased the amylase activity. Changes in amylase activity in leaves of *Aesculus hippoeastanum* have also been noted [2, 3]

Though in earlier reports [15] it was suggested that synthesis of amylase did not depend on the dose of γ-irradiation within 5 – 200 kR; the findings of present work and survey of many works clearly states that activity of amylase gets affected even at very low dose of 1 kR.

In conclusion it can be said that seedlings from the soaked seeds showed more inhibitory response than dry seeds when irradiated with γ-radiation. Seedling growth was affected due to inhibition in amylase activity.

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