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Effect of γ ray irradiated pollen technique on fruit growth in Citrus

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Abstract

Citrus is one of the most economically important fruit crops in the world, greatly valued as table fruit as well as in the processing industry. Among the different citrus fruits, pummelo plays a major role in the processing industry. However, the presence of a large number of seeds in pummelo greatly hinders processing quality. Therefore, induction of seedlessness in pummelo is one of the major goals around the world. Currently, irradiated pollen technique emerges as a novel tool for induction of seedlessness in any fruit. However, reduction of fruit size is a great concern in irradiated pollen technique. Hence, an experiment was conducted to study the effect of gamma ray irradiated pollen technique on fruit growth in citrus using pummelo (*C. grandis* (L.) Osb.) as seed parent while sweet orange (*Citrus sinensis* (L.) Osb.) *cv.* 'Mosambi' and sweet lemon (*C. limetta* Risso) as pollen sources. Irradiation of pollen grains was performed at 50, 100, 200, 300 and 400 Gray (Gy) by using ^{60}Co gamma ray and the experiment was laid out in complete randomized design with 5 replications. With the increased doses of irradiation, fruit growth in terms of length, diameter, weight and volume differed significantly throughout the period of observation starting from 35 days after pollination to till maturity. At maturity, maximum fruit length (387.70 mm), weight (590.27 g) and volume (885.27 cc) were recorded in *C. grandis* × *C. limetta* crosses at control while minimum fruit length (129.74 mm) and weight (211.54 g) were observed in *C. grandis* × *C. sinensis* crosses at 300 Gy. There was no fruit retention till maturity in either cross combination at 400 Gy. Among two different cross combinations, with the increased dose of irradiation, the rate of reduction in fruit growth in terms of length and weight as compared to respective control was much higher in *C. grandis* × *C. sinensis* crosses. However, for the production of marketable size fruits (not more than 300 g), irradiation of either pollen parent at 200-300 Gy was found to be the best while, doses below 200 Gy caused the production of fruits weighing more than 400 g, which do not have good market value.

Key words: Citrus, irradiation, fruit size, fruit volume, fruit weight, gamma-ray.

Introduction

Citrus are one of most important fruit crops in the world in economic terms (Benkeblia and Tennant, 2008). The global trade of citrus fruit demands high cosmetic and organoleptic characteristics. Currently, seedlessness emerged as one of the main criteria for better marketing of citrus fruits and one of the most important characteristics with respect to quality of fruits for consumption (Gambetta *et al.*, 2013). However, the presence of a large number of seeds in citrus fruits particularly in pummelo is a big hindrance for consumer acceptability. Seedlessness is a desirable characteristic for both fresh and processed citrus markets. Therefore, development of seedless cultivars has become a major goal for citrus breeders around the world (Ye *et al.*, 2009). Over the past few decades, great achievements have been gained in seedless breeding programme in different fruits like grape (Ji *et al.*, 2013), guava (Ulukapi and Nasircilar, 2015), mango (Usman *et al.*, 2001), citrus (Raza *et al.*, 2003 and Bermejo *et al.*, 2011) and in others by employing different approaches like polyploidy breeding, mutation breeding, irradiated pollen technique, application of PGR, embryo rescue technique etc. Among the all proposed strategies, irradiated pollen technique has emerged as one of the most novel tools for inducing parthenogenesis in citrus. Moreover, irradiation not only affects the seed formation in the fruits, but also favours early ripening, lowers the acidity content and improves the quality parameters of the fruit (Bermejo *et al.*, 2011). Gamma rays are commonly used for parthenogenesis in citrus because of their simple application, good penetration, reproducibility, high mutation frequency and less disposal problems (Chahal and Gosal, 2002).

However, the major drawback of irradiated pollen technique is the reduction of fruit size in terms of fruit weight and shape (Bermejo *et al.*, 2011 and Goldenberg *et al.*, 2014). On the other hand, in citrus and other woody perennials, along with other quality parameters, fruit size plays a vital role for consumer and industrial acceptability. Hence, it is the urgent need of the hours to study the impact of gamma irradiated pollen technique on fruit growth in citrus. As per our knowledge, no study has been conducted till date concerning the effect of gamma irradiation doses on fruit growth in citrus. Therefore, the present study aimed to examine the effect of gamma irradiated pollen technique on fruit growth in *Citrus*.

Materials and methods

Based on our previous year's study on standardization of gamma ray irradiation doses and pollen parents for pollen viability, fruit set (Kundu *et al.*, 2014) and seed development in citrus, two *Citrus* species, *viz.*, sweet orange (*Citrus sinensis* (L.) Osb.) *cv.*

‘Mosambi’ and sweet lemon (*C. limetta* Risso) were selected as pollen sources for the experiment. Pummelo (*C. grandis* (L.) Osb.), a monoembryonic diploid *Citrus* species, was selected as seed parent.

Irradiation of pollen grains of these two pollen parents was performed at 50, 100, 200, 300 and 400 Gray (Gy) by using ^{60}Co gamma rays (NRL, IARI, New Delhi, India). Thereafter, emasculation and pollination of *C. grandis* was carried out with irradiated pollen grains followed by bagging and labeling according to the method described by Kundu *et al.* (2014).

Data on fruit growth with respect to fruit length, diameter, volume and weight for each exposure doses were recorded at 20, 35, 50, and 65 days after pollination (DAP) and again at harvesting. Fruit length and diameter were measured with the help of digital vernier calliper, while fruit weight and volume were recorded by weighing the sample on digital weighing balance and by water displacement method, respectively.

The experiment was laid out in complete randomized design with 5 replications. Statistical analysis was performed using statistical analysis software (SAS 9.3; SAS Institute, Cary, NC, USA) and the mean values were compared through Tukey’s Honest Significant Difference (THSD) Test.

Results and discussion

Fruit growth in terms of length, diameter, weight and volume showed a linear pattern of growth during the entire period of observation starting from 20 DAP till maturity. Irrespective of irradiation doses, fruit length did not differ significantly up to 35 DAP and the highest fruit length was recorded in *C. grandis* × *C. limetta* crosses as compared to *C. grandis* × *C. sinensis* crosses (Table 1). Similar pattern was also observed for fruit weight and volume during the entire period of observation while fruit diameter differed non-significantly up to 50 DAP with higher values in *C. grandis* × *C. limetta* crosses at 65 DAP (113.09 mm) and in *C. grandis* × *C. sinensis* crosses at maturity (269.88 mm) (Table 2). These differences might be due to differences in genotypes and irradiation doses during the entire period of fruit growth starting from 35 DAP. Regardless of pollen parent, fruit length, diameter and volume showed non-significant difference for varying level of irradiation doses at 20 DAP but from 35 DAP to till maturity, they decreased gradually with the increasing concentrations of irradiation doses. We did not get a single fruit on the tree at maturity for 400 Gy treatments in either cross combinations. This is a novel finding in this study and it might be due to higher sensitivity of pollens grains of both the pollen parents to higher irradiation doses, resulting in degeneration of pollen tubes within the style at latter half of pollen tube growth or pseudo-fertilization of the ovary. This may cause abscission of fruit at the late phase of fruit growth before reaching to maturity (Musial and Przywara, 1998). However, at maturity, maximum fruit length (387.23 mm), diameter (394.74 mm), weight (576.96 g) and volume (869.17 cc) was recorded in crosses with non-irradiated pollens and the fruit size was decreased gradually with the increased doses of irradiation and it was recorded minimum (132.12 mm, 130.59 mm, 214.99 g and 252.17 cc, respectively) in the crosses where highest level of irradiation (300 Gy) was used to treat the pollen grains. Perusal of interaction indicated significant

Table 1. Effect of pollen parent and gamma ray irradiation on fruit length in *Citrus grandis*

Treatment	Fruit length (mm)				
	20 DAP	35 DAP	50 DAP	65 DAP	Maturity
Pollen parent (P)					
C. sinensis	27.30^{a}	39.07^{a}	56.62^{b}	102.93^{b}	261.69^{b}
C. limetta	26.91^{a}	39.81^{a}	59.25^{a}	111.89^{a}	266.80^{a}
Irradiation dose (I)					
Control	26.68^{a}	46.01^{a}	67.52^{a}	147.17^{a}	387.23^{a}
050 Gy	25.40^{a}	44.13^{ab}	64.31^{ab}	129.41^{b}	324.35^{b}
100 Gy	26.24^{a}	40.61^{bc}	60.07^{bc}	119.88^{c}	302.20^{c}
200 Gy	27.12^{a}	38.14^{cd}	56.04^{cd}	98.78^{d}	176.32^{d}
300 Gy	28.85^{a}	35.14^{de}	52.05^{de}	83.19^{e}	132.12^{e}
400 Gy	28.36^{a}	32.59^{e}	47.61^{e}	66.03^{f}	-
P × I Interaction					
C. sinensis					
Control	27.03^{a}	45.01^{ab}	66.00^{ab}	148.22^{a}	386.76^{a}
050 Gy	26.48^{a}	43.82^{abc}	63.58^{abc}	126.97^{b}	329.40^{b}
100 Gy	28.00^{a}	40.21^{bcd}	59.10^{bcde}	115.88^{cd}	298.20^{c}
200 Gy	25.37^{a}	38.07^{cde}	53.96^{defg}	89.09^{e}	164.36^{e}
300 Gy	28.67^{a}	35.20^{de}	51.04^{fgh}	72.92^{f}	129.74^{f}
400 Gy	28.25^{a}	32.08^{e}	46.02^{h}	64.49^{f}	-
C. limetta					
Control	26.33^{a}	47.00^{a}	69.04^{a}	146.11^{a}	387.70^{a}
050 Gy	24.33^{a}	44.45^{abc}	65.04^{abc}	131.84^{b}	319.30^{b}
100 Gy	24.49^{a}	41.00^{abcd}	61.04^{bcd}	123.88^{bc}	306.20^{c}
200 Gy	28.87^{a}	38.21^{cde}	58.12^{cdef}	108.46^{d}	186.29^{d}
300 Gy	29.03^{a}	35.09^{de}	53.06^{efgh}	93.46^{e}	134.50^{f}
400 Gy	28.47^{a}	33.10^{e}	49.20^{gh}	67.57^{f}	-

Table 2. Effect of pollen parent and gamma ray irradiation on fruit diameter in *Citrus grandis*

Treatment	Fruit diameter (mm)				
	20 DAP	35 DAP	50 DAP	65 DAP	Maturity
Pollen parent (P)					
C. sinensis	24.84^{a}	42.40^{a}	57.86^{a}	105.78^{b}	269.88^{a}
C. limetta	24.62^{a}	43.70^{a}	59.74^{a}	113.09^{a}	264.02^{b}
Irradiation dose (I)					
Control	25.10^{a}	51.57^{a}	69.14^{a}	150.32^{a}	394.74^{a}
050 Gy	22.33^{a}	48.54^{ab}	65.05^{ab}	132.86^{b}	328.61^{b}
100 Gy	24.22^{a}	44.05^{bc}	59.99^{bc}	120.90^{c}	298.61^{c}
200 Gy	23.89^{a}	41.08^{cd}	56.89^{cd}	99.90^{d}	182.21^{d}
300 Gy	26.70^{a}	37.99^{de}	52.31^{de}	85.44^{e}	130.59^{e}
400 Gy	26.15^{a}	35.08^{e}	49.41^{e}	67.22^{f}	-
P × I Interaction					
C. sinensis					
Control	24.51^{a}	51.12^{ab}	68.06^{a}	152.08^{a}	396.88^{a}
050 Gy	23.29^{a}	48.08^{abc}	64.06^{abc}	129.35^{bc}	334.18^{b}
100 Gy	26.09^{a}	44.02^{bcd}	58.02^{bcd}	115.02^{d}	291.94^{e}
200 Gy	22.43^{a}	40.16^{def}	55.98^{cde}	92.00^{e}	182.07^{f}
300 Gy	26.64^{a}	37.00^{def}	52.12^{de}	78.01^{f}	144.32^{g}
400 Gy	26.09^{a}	34.04^{f}	48.90^{e}	68.24^{g}	-
C. limetta					
Control	25.68^{a}	52.02^{a}	70.22^{a}	148.56^{a}	392.60^{a}
050 Gy	21.37^{a}	49.00^{abc}	66.03^{ab}	136.36^{b}	323.04^{c}
100 Gy	22.36^{a}	44.08^{bcd}	61.95^{abc}	126.78^{c}	305.28^{d}
200 Gy	25.35^{a}	42.00^{cde}	57.80^{bcd}	107.80^{d}	182.34^{f}
300 Gy	26.75^{a}	38.98^{def}	52.50^{de}	92.86^{e}	116.86^{h}
400 Gy	26.20^{a}	36.12^{ef}	49.92^{de}	66.20^{g}	-

Values indicate mean of five replicates. Different letters in the same column indicate significant differences at $P \leq 0.05$ (Tukey’s Honest Significant Difference Test); DAP: days after pollination.

difference in fruit length, diameter, weight and volume at 35 DAP onwards. It decreased gradually with increasing concentration of exposure doses. At maturity, higher fruit length (387.70 mm), weight (590.27 g) and volume (885.27 cc) were recorded in *C. grandis* × *C. limetta* crosses in control while less fruit length (129.74 mm) and weight (211.54 g) were in *C. grandis* × *C. sinensis* crosses at 300 Gy (Table 1, 3 and 4). The less volume was measured in both the crosses at 300 Gy (Table 4). Fruits of *C. grandis* × *C. limetta* crosses at 300 Gy had less diameter (116.86 mm) followed by fruits of *C. grandis* × *C. sinensis* crosses at 300 Gy (144.32 mm). At 35 DAP, although, the rate of reductions of fruit length with the gradual increases of irradiation doses was much higher in *C. grandis* × *C. limetta* crosses but from 50 DAP onwards these reduction was much higher in *C. grandis* × *C. sinensis* crosses (Table 1).

At maturity, the reduction in fruit length at 300 Gy as compared to respective control was 66.45% in *C. grandis* × *C. sinensis* crosses while it was 65.31% in *C. grandis* × *C. limetta*. Similar pattern was also observed for fruit weight during the entire period of observation. This might be due to higher resistance of *C. limetta* pollen to irradiation doses as compared to *C. sinensis* pollen, as the response of pollen grains to irradiation doses is genotype specific (Kundu *et al.*, 2014). However, with the increase of time, as the fruit reached towards maturity, the rate of increase of fruit length slowed down at faster rate with the increasing concentration of irradiation doses. In *C. grandis* × *C. sinensis* crosses in control, the rate of increase of fruit length at 35, 50, 65 DAP and at maturity was 66.52, 46.63, 124.58 and 160.94% higher than fruit length of respective previous date of observations but the rate of increase at the same time period at 300 Gy was only 22.78, 45, 42.87 and 77.92% higher as compared to respective previous date, respectively while at 400 Gy, it was only 13.56, 43.45 and 40.13% higher for 35, 50, 65 DAP than respective previous observations respectively. Similar pattern of slow increase of fruit length with increasing time period and increasing concentration of irradiation doses was also observed in *C. grandis* × *C. limetta* crosses. Likewise, the rate of increase of fruit diameter, weight and volume was also slowed down progressively as compared to respective previous observations, with the increasing concentration of irradiation doses as the fruits reached towards maturity. These might be because of increasing sensitivity of the pollen grains to gradual increase of irradiation doses, as the response of pollen grains to irradiation doses is also a dose dependent phenomenon (Kundu *et al.*, 2014). It results poor growth of pollen tube within the style and subsequent degeneration of these tubes before reaching to the ovarian wall, which ultimately disturbs double fertilization, and subsequently the development and interactions of embryo and endosperm as envisaged by rapid decrease in seed content within fruit due to higher doses of irradiation.

In citrus, fruit size, weight, and volume are positively related to number of seeds within fruits. Numerous studies have demonstrated plant hormones *viz.,* auxins and gibberellins having a big role in fruit growth and development (Pandolfini, 2009). Pollen grains are the good source of auxins (Feng *et al.*, 2006); developing pollen tubes also stimulates the auxins production within the style (Wu *et al.*, 2008) and into the ovules (Dorcey *et al.*, 2009); after fertilization additional auxins supply comes from

Table 3. Effect of pollen parent and gamma ray irradiation on fruit weight in *Citrus grandis*

Treatment	Fruit weight (g)				
	20 DAP	35 DAP	50 DAP	65 DAP	Maturity
Pollen parent (P)					
C. sinensis	9.20^{a}	37.50^{a}	77.23^{b}	148.95^{b}	398.43^{b}
C. limetta	8.75^{a}	37.79^{a}	79.05^{a}	155.14^{a}	408.35^{a}
Irradiation dose (I)					
Control	8.43^{ab}	51.42^{a}	100.55^{a}	195.49^{a}	576.96^{a}
050 Gy	7.44^{b}	47.14^{a}	92.14^{b}	183.19^{b}	505.69^{b}
100 Gy	7.87^{b}	41.91^{b}	81.12^{c}	169.13^{c}	447.07^{c}
200 Gy	8.33^{b}	33.62^{c}	70.50^{d}	139.96^{d}	272.25^{d}
300 Gy	11.40^{a}	27.63^{d}	64.58^{e}	117.37^{e}	214.99^{e}
400 Gy	10.37^{ab}	24.16^{d}	59.96^{f}	107.12^{f}	-
P × I Interaction					
C. sinensis					
Control	8.55^{ab}	51.02^{a}	99.20^{ab}	192.48^{ab}	563.65^{b}
050 Gy	8.08^{ab}	47.28^{ab}	93.16^{bc}	180.19^{bcd}	500.46^{c}
100 Gy	9.24^{ab}	41.00^{bc}	82.02^{d}	165.34^{d}	442.87^{d}
200 Gy	7.80^{ab}	33.20^{cd}	69.08^{e}	135.80^{e}	273.65^{e}
300 Gy	11.50^{a}	28.22^{de}	62.02^{fg}	115.49^{f}	211.54^{f}
400 Gy	10.03^{ab}	24.30^{e}	57.92^{g}	104.38^{f}	-
C. limetta					
Control	8.31^{ab}	51.82^{a}	101.90^{a}	198.50^{a}	590.27^{a}
050 Gy	6.80^{ab}	47.00^{ab}	91.12^{c}	186.18^{abc}	510.92^{c}
100 Gy	6.50^{b}	42.82^{b}	80.22^{d}	172.92^{cd}	451.26^{d}
200 Gy	8.87^{ab}	34.04^{cd}	71.92^{e}	144.12^{e}	270.85^{e}
300 Gy	11.31^{ab}	27.03^{de}	67.14^{ef}	119.24^{f}	218.44^{f}
400 Gy	10.71^{ab}	24.02^{e}	62.00^{fg}	109.86^{f}	-

Table 4. Effect of pollen parent and gamma ray irradiation on fruit volume in *Citrus grandis*

Treatment	Fruit volume (cc)				
	20 DAP	35 DAP	50 DAP	65 DAP	Maturity
Pollen parent (P)					
C. sinensis	15.58^{a}	57.72^{b}	121.11^{b}	215.26^{a}	563.33^{b}
C. limetta	14.11^{a}	60.24^{a}	128.12^{a}	219.28^{a}	577.20^{a}
Irradiation dose (I)					
Control	14.50^{a}	79.06^{a}	151.51^{a}	330.22^{a}	869.17^{a}
050 Gy	13.40^{a}	73.05^{b}	141.09^{b}	273.06^{b}	717.00^{b}
100 Gy	14.17^{a}	62.67^{c}	126.75^{c}	253.17^{c}	635.33^{c}
200 Gy	14.90^{a}	52.18^{d}	115.21^{d}	191.17^{d}	377.67^{d}
300 Gy	16.40^{a}	46.81^{e}	109.42^{e}	137.17^{e}	252.17^{e}
400 Gy	15.70^{a}	40.11^{f}	103.72^{f}	118.83^{f}	-
P × I Interaction					
C. sinensis					
Control	15.00^{a}	76.00^{ab}	145.82^{b}	326.11^{a}	852.67^{b}
050 Gy	14.50^{a}	72.02^{bc}	134.00^{c}	272.45^{b}	705.33^{d}
100 Gy	16.00^{a}	61.00^{de}	124.51^{de}	244.33^{c}	625.33^{f}
200 Gy	14.00^{a}	53.06^{ef}	113.22^{fg}	191.33^{d}	379.33^{g}
300 Gy	17.50^{a}	46.12^{fg}	107.80^{gh}	138.67^{e}	254.00^{h}
400 Gy	16.50^{a}	38.12^{h}	101.38^{h}	118.67^{e}	-
C. limetta					
Control	14.00^{a}	82.12^{a}	157.20^{a}	334.33^{a}	885.67^{a}
050 Gy	12.30^{a}	74.08^{b}	148.18^{b}	273.67^{b}	728.67^{c}
100 Gy	12.33^{a}	64.34^{cd}	128.99^{cd}	262.00^{bc}	645.33^{e}
200 Gy	15.80^{a}	51.30^{f}	117.20^{ef}	191.00^{d}	376.00^{g}
300 Gy	15.30^{a}	47.50^{fg}	111.04^{fg}	135.67^{e}	250.33^{h}
400 Gy	14.90^{a}	42.10^{gh}	106.06^{gh}	119.00^{e}	-

Values indicate mean of five replicates. Different letters in the same column indicate significant differences at $P \leq 0.05$ (Tukey's Honest Significant Difference Test); DAP: days after pollination.

the fertilized ovules (Moctezuma, 1999) while the developing endosperm also produce higher quantity of auxins (Srivastava, 2003) resulting better growth and development of the fruits at the initial stage of fruit growth and isodiameric enlargement of the cells surrounding the ovary wall (Moctezuma, 1999) resulting enlargement of the ovary *i.e.* fruit development. But in our study, due to the poor response of pollen grains to increasing concentration of irradiation doses and subsequent pseudo-fertilization of ovary, auxins production might be deprived, resulting poor growth of the fruits in terms of size, weight and volume during the early stage of fruit growth.

For the production of marketable size fruits (not more than 300 g) irradiation of either pollen parent at 200-300 Gy was found to be the best while, doses below 200 Gy cause the production of fruits weighing more than 400 g, which do not have good marketability. However, irradiation dose of 400 Gy was found lethal for all the citrus genotypes evaluated as pollen parent under the study, resulting no fruit retention till maturity. Hence, Irradiation doses beyond 300 Gy could not be used for induction of seedlessness in Citrus.

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