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New Method of Producing Diploid Seedless Watermelon Fruit

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Abstract

We found that diploid seedless watermelon can be produced by pollination with partially functional pollen which was irradiated with soft X-rays at the doses of 800 to 1000 Gy. The diploid seedless fruit was almost similar to the normal fruit in size, shape, color, rind thickness, sugar content, days to maturity from pollination and rate of fruit set. The number of empty seeds in the diploid seedless fruit varied among the cultivars used in this study. No correlation was found between the number of empty seeds in seedless fruit and the number of normal seeds in normal fruit. The pollen tube of the pollen irradiated with soft X-rays penetrated normally into the synergid and sperm cells were discharged. Subsequently, the egg nucleus and sperm nucleus became attached to each other in the egg cell and a globular embryo was formed. However, the embryo failed to differentiate to organ tissues and degenerated.

Discipline: Horticulture
Additional key words: *Citrullus lanatus,* embryo, parthenocarpy, pollination, X-ray

Introduction

Seedless watermelon fruits are popular with consumers because it is very easy to eat them. Presently, seedless watermelons are produced by utilizing triploid plants. This method was developed about 50 years ago[3,4,10]. Seedless watermelon fruits have also been produced using plant growth regulators[2,8,9,12]. However, the cost of triploid seedless watermelons is very high for seed production and plant growth regulators cannot be used from safety's point of view. Because the current production methods of seedless watermelon are associated with shortcomings[4,5,13], it is necessary to develop a new method to produce seedless watermelon fruits which would be more convenient for and acceptable to farmers. We eventually succeeded in developing a more practical method than the current methods. In our method, only pollination with irradiated pollen is required. These seedless watermelons can grow by using ordinary cultivation practices. In this paper, the method for producing seedless watermelons will be discussed in detail.

Materials and methods

Effect of soft X-ray dose on number of seeds and fruit sets

1. Fujihikari TR and Benikodama cultivars of watermelon (F_1 hybrid) were sown on April 4, 1997. Male flowers were harvested in the morning when they bloomed. The prepared male flowers were irradiated with soft X-rays (Soft X-ray Unit OM-60R, OHMIC Ltd., Tokyo, Japan) at 11.1 Gy・min^{-1}. They were irradiated with a dose of 0 (unirradiated, as the control), 100, 200, 400, 800 or 1000 Gy. Female flowers were covered with cellophane bags to prevent contact with pollen carried by insects before or after the bloom. Mature fruits were harvested at about 35 days for 'Benikodama' and 38 days after pollination for 'Fujihikari TR'.
2. 'Fujihikari TR' and 'Benikodama' were sown on November 13, 1997 and February 26, 1998, respectively. Male flowers were irradiated with 800 Gy on the blooming day. After irradiation, the pollen was used for investigations of the fruit set rate.

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Characteristics of fruits produced by pollen irradiation with soft X-rays

Eleven cultivars were compared (Table 3). They were sown on August 11, 1997. Five plants per cultivar were used in the treatments. The blooming male flowers of 'Fujihikari TR' were irradiated with 800 Gy of soft X-rays and immediately used to pollinate each watermelon plant. Unirradiated pollen of 'Fujihikari TR' was used as the control. After harvest, fruit weight, fruit shape, flesh color, rind thickness, sugar content (soluble solids content) and days to maturity were investigated. For the fruit characteristics, the mean differences between normal fruit and seedless fruit were determined by the *t* test.

Abortion of embryo following pollination with pollen irradiated with soft X-rays in watermelon

'Fujihikari TR' was sown on March 3, 1998. The male flowers were collected in the morning of anthesis. The blooming male flowers were irradiated with a 800 Gy dose of soft X-rays and were immediately used for pollination. A minimum number of 5 fruits was selected at random at 0 to 20 days after pollination with pollen irradiated with soft X-rays or unirradiated pollen and rapidly fixed with a FAA solution (formalin-acetic acid-alcohol). The fixed specimens were dehydrated in a graded t-butanol series, embedded in paraffin, sliced into 10 µm-thick serial sections. The sections were stained with haematoxylin and safranin, or with haematoxylin, safranin and fast green. Coverslips were put on the stained sections using balsam prior to microscopic examination.

Results

Effect of soft X-ray dose on number of seeds and fruit sets

The number of normal seeds was significantly reduced when female flowers were pollinated with irradiated pollen (Table 1). In 'Benikodama', normal seeds were not observed at 800–1000 Gy. About 250–350 empty seeds per fruit were observed at 100–1000 Gy. In 'Fujihikari TR' irradiated with 800–1000 Gy, only empty seeds were produced (Fig. 1). The numbers of empty seeds were about 70–90 at 800–1000 Gy. 'Benikodama' had more empty seeds than 'Fujihikari TR'.

No differences were detected in the fruit set between pollen irradiated with soft X-rays and unirradiated pollen in both 'Benikodama' and 'Fujihikari TR' (Table 2).

Characteristics of fruits produced by pollen irradiated with soft X-rays

The weight of the seedless fruit was similar to that of the normal fruit for each cultivar except for 'Red Seeded 3b' (Table 3). Seedless 'Tetsukabuto' had a deeper flesh color than the control. The rind of 'Red Seeded 3b' and 'Tenryu No. 2' fruit was thinner in the seedless fruits than in the normal fruit, but in 'Kobyo' and 'Gengobe' the tendency was reversed. The seedless fruit of 'Gengobe' had a slightly higher sugar content than the normal fruit, while the other cultivars did not show any differences. Days to maturity of seedless fruit were similar to those of the normal fruit. Thus, there was no consistent relationship between these fruit characteristics and irradiation with soft X-rays.

Table 1. Relationship between soft X-ray irradiation dose and number of seeds in watermelon fruit

Cultivar	Soft X-ray (Gy)	No. of normal seeds	No. of empty seeds: Total	(white	color)	Total no. of seeds
Benikodama	0	346 ± 33	55 ± 11	(55	0)	401 ± 39
	100	93 ± 21	294 ± 16	(233	61)	387 ± 15
	200	36 ± 6	341 ± 17	(307	34)	377 ± 20
	400	2 ± 2	305 ± 24	(295	10)	307 ± 24
	800	0 ± 0	261 ± 19	(261	0)	261 ± 19
	1000	0 ± 0	285 ± 26	(284	1)	285 ± 26
Fujihikari	0	623 ± 39	114 ± 43	(114	0)	737 ± 30
	100	279 ± 54	206 ± 76	(108	98)	484 ± 122
	200	48 ± 33	255 ± 68	(175	80)	302 ± 100
	400	31 ± 7	182 ± 7	(106	76)	213 ± 13
	800	0 ± 0	89 ± 7	(82	7)	89 ± 7
	1000	0 ± 0	78 ± 13	(65	12)	78 ± 13

Values are means ± SE.

Table 2. Effect of irradiated pollen on fruit set of watermelon

Cultivar	X-ray (Gy)	No. of pollinated female flowers	No. of fruit sets	Fruit set (%)
Benikodama	0	51	20	39.2
	800	46	20	43.5
Fujihikari TR	0	54	35	64.8
	800	60	40	66.7

In the seedless fruit, only empty seeds were found, but their frequency ranged widely. 'Tabata' showed the largest number of empty seeds; its seedless fruit contained 619 empty seeds and more than 50% of all the empty seeds were black or brown. The normal fruit of 'Crimson Sweet' contained 434 seeds and the seedless fruit had 7 empty seeds. The seedless fruit of 'Klekley Sweet' and 'Tenryu No. 2' had 26 and 31 empty seeds, respectively.

Abortion of embryo following pollination with pollen irradiated with soft X-rays in watermelon

Using unirradiated or irradiated pollen, the pollen tube penetrated into one of the two synergids one day after pollination and sperm nuclei were normally discharged. At 2 to 3 days after pollination, a sperm nucleus and egg nucleus were attached to each other in the egg cell (Figs. 2a, 3a). The other sperm nucleus in the same embryo sac was adjacent to the 2 polar nuclei. Proembryo and globular-shaped embryo were formed 5 to 7 days after pollination with unirradiated pollen (Fig. 2b), and a heart-shaped embryo was observed at about 8 days (Fig. 2c). The embryo differentiated into cotyledons, radicle, epicotyl, and hypocotyl by days 12 to 16, and subsequently developed into a mature embryo. In contrast, there was a slight delay in the development of the proembryo and globular-shaped embryo following pollination with irradiated pollen compared with pollination

Table 3. Characteristics of watermelon fruits developed using pollen irradiated with soft X-rays

Cultivar	Fruit type	Fruit weight (kg)	Flesh shape[a)]	Flesh color[b)]	Thickness of rind (mm)	Brix value (%)	Days to maturity[c)] (d)	No. of seeds		
								Normal	Empty	Total
Crimson Sweet	normal	2.9	2.9	R 3.4	11.1	9.2	49.7	337	97	434 ± 36[d]
	seedless	3.1	2.9	R 3.2	11.4	9.1	48.5	0	7	7 ± 6
Gengobe	normal	1.9	1.1	R 1.9	8.4	8.1	45.7	320	26	346 ± 28
	seedless	1.8	1.1	R 2.0	9.5**	8.7**	44.4	0	243	243 ± 23
Kurume No.1	normal	1.9	1.1	R 3.0	6.5	10.9	38.5	175	2	177 ± 34
	seedless	1.6	1.1	R 3.1	6.2	10.7	38.3	0	100	100 ± 24
Miyako No.3	normal	2.1	1.0	R 2.8	8.7	10.0	42.0	420	82	502 ± 59
	seedless	2.3	1.0	R 2.7	8.5	9.5	41.5	0	200	200 ± 29
Tetsukabuto	normal	2.5	1.1	R 2.6	10.0	9.4	42.6	317	3	320 ± 48
	seedless	2.0	1.1	R 3.0*	9.3	9.0	40.7	0	146	146 ± 43
Klekley Sweet	normal	4.2	1.4	R 3.5	11.0	10.8	50.3	451	7	458 ± 81
	seedless	4.3	1.5	R 3.3	10.8	10.4	49.0	0	26	26 ± 12
Kobyo	normal	3.1	1.7	R 2.4	11.2	9.9	49.0	334	130	464 ± 86
	seedless	2.8	1.8	R 2.6	13.3**	9.7	50.2	0	62	62 ± 35
Koryo 200	normal	2.7	1.0	R 3.0	9.4	10.0	42.0	305	85	390 ± 78
	seedless	2.9	1.1	R 3.1	9.4	10.7	42.3	0	70	70 ± 22
Red Seeded 3b	normal	2.5	1.0	YW 1.0	16.9	1.6	51.0	413	5	418 ± 26
	seedless	2.0*	1.0	YW 1.0	13.7**	1.6	51.0	0	87	87 ± 23
Tabata	normal	3.3	1.1	R 3.0	10.4	9.0	47.7	523	187	710 ± 34
	seedless	3.6	1.1	R 3.0	10.9	8.9	47.3	0	619	619 ± 44
Tenryu No.2	normal	2.9	1.1	R 3.2	10.9	9.2	41.7	297	66	363 ± 84
	seedless	2.6	1.1	R 3.0	8.6**	9.4	42.7	0	31	31 ± 12

a): Fruit shape index was expressed as the ratio of height relative to width.
b): R, red; W, white; YW, yellowish white. 1(light color)–5(deep color).
c): From pollination until harvest.
d): Mean ± SE.
*, ** Significant between normal fruit and seedless fruit at P<0.05 or 0.01, respectively.

Fig. 1.

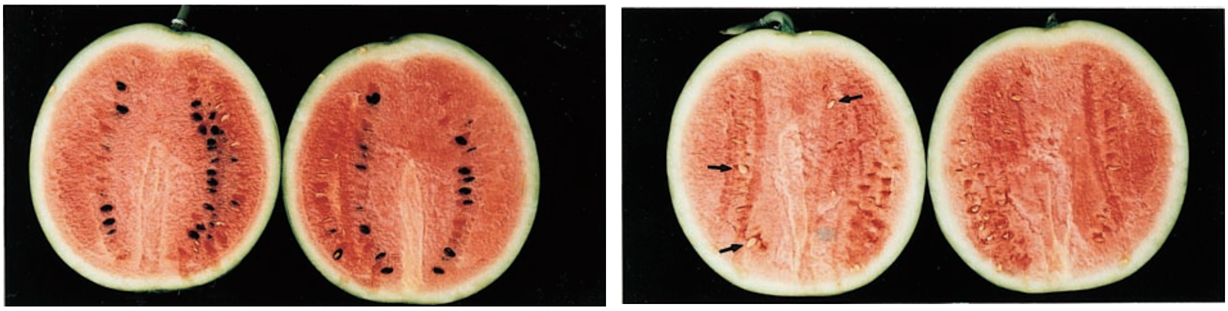


Fig. 2.

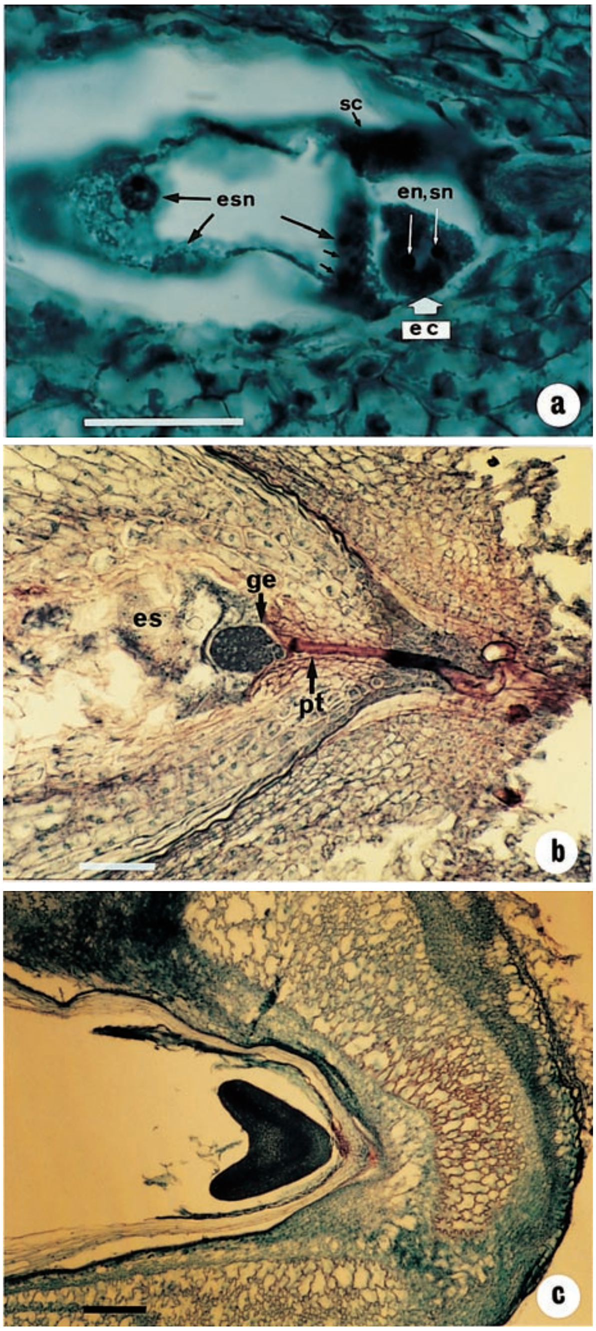
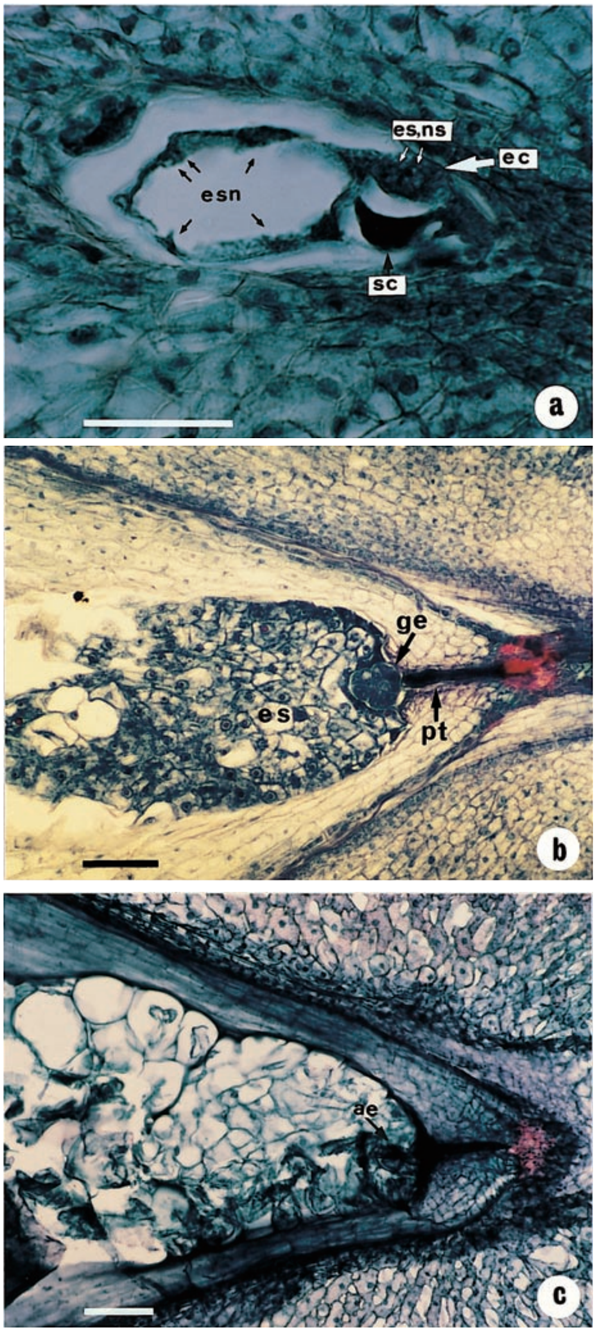


Fig. 3.



with unirradiated pollen (Fig. 3b). The shape of the globular embryo after pollination with irradiated pollen was deformed and there were fewer cells than in a normal embryo. The embryo failed to differentiate organ tissues and degenerated 10 to 20 days after pollination (Fig. 3c).

Discussion

Pollen irradiated with soft X-rays did not affect the fruit set. We observed that the germination rate of the pollen treated with 800 Gy was almost the same as that of the control[7]. These results indicate that irradiated pollen can be used for producing seedless watermelon.

Triploids tend to show a longer growing period and a later maturity than diploid ones[5], and they sometimes display developmental defects such as hollow hearts and thick rinds. In contrast, such defects were not observed in the seedless watermelon produced by pollination with pollen irradiated with soft X-rays because diploid seedless watermelon can be produced by using common diploid cultivars.

The occurrence of empty seeds had been recognized long time ago in triploid watermelon. In diploid watermelons, when seedless watermelons are produced from cultivars with a few normal seeds, the fruits contain a few empty seeds. However, when seedless watermelons are produced from cultivars with very few seeds, the number of empty seeds may not necessarily decrease. Also, there was a high correlation between the size of normal seeds and empty seeds[7]. We sometimes observed colored empty seeds in seedless fruits. A colored empty seed is produced when the seed coat is black or brown. Therefore, it is preferable to use cultivars with a white seed coat to produce seedless watermelon. The number of empty seeds is influenced by the temperature and also the color of the empty seeds tends to become deeper when the temperature is high[11]. It is necessary to analyze the effect of environmental factors on seed formation.

In a previous study of watermelon, discharge of sperm nuclei occurred 2 days after pollination with normal pollen[1]. In our study, a similar finding was observed after pollination with irradiated pollen. Embryonic development in watermelon was reported to occur in pollinated, but not in unpollinated[1] or auxin-induced parthenocarpic ovules[6]. We assumed that fertilization following sperm cell discharge occurs with pollen irradiated with soft X-rays as well as with unirradiated pollen. It is generally recognized that irradiation of pollen with γ- or X-rays may induce genetic abnormalities. The abortion of embryos associated with pollination with pollen irradiated with soft X-rays may be due to chromosomal abnormalities induced by soft X-rays in the generative nucleus. It is thus necessary to determine whether all or part of the chromosomes in sperm nuclei are involved in nuclear fusion.

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Fig. 1. Diploid seedless watermelon produced by pollen irradiated with soft X-rays
Japanese F_1 cultivar Fujihikari TR. Left: Normal fruit. Right: Seedless fruit. Arrows indicate empty seeds.

Fig. 2. Process of fertilization and embryo formation with unirradiated pollen
(a): Section of embryo sac on day 3 (72 h) after pollination with unirradiated pollen, showing an egg nucleus and sperm nucleus attached to each other in the egg cell and endosperm nuclei. Endosperm nuclei have expanded in the embryo sac. (b): Section of embryo sac on day 5, showing globular embryo. (c): Section of embryo sac on day 8, showing heart-shaped embryo. ec, egg cell; en, egg nucleus; es, endosperm; esn, endosperm nuclei; ge, globular embryo; pt, pollen tube; sc, synergid cell; sn, sperm nucleus. Horizontal bar: (a), 25 µm; (b), 50 µm; (c), 100 µm.

Fig. 3. Process of fertilization and embryo formation with irradiated pollen
(a): Section of embryo sac on day 3 (72 h) after pollination with irradiated pollen, showing an egg nucleus and sperm nucleus attached to each other in the egg cell. Endosperm nuclei have spread in the embryo sac. (b): Section of embryo sac on day 7, showing globular embryo. (c): Section of embryo sac on day 18, showing an aborted embryo. ae, aborted embryo; ec, egg cell; en, egg nucleus; es, endosperm; esn, endosperm nuclei; ge, globular embryo; pt, pollen tube; sc, synergid cell; sn, sperm nucleus. Horizontal bar: (a), 25 µm; (b, c), 50 µm.

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