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ORIGINAL ARTICLE

Effects of gamma ray sources and irradiation doses on the haploidization frequency of citron watermelon (*Citrullus lanatus* var. *citroides*)

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Abstract

Drought is widely regarded as the most significant abiotic stressor affecting modern agricultural production. Sweet watermelon (*Citrullus lanatus* var. *lanatus*), one of the world's most cultivated vegetable species, lacks drought resistance and must be grown in dry and semi-arid regions by grafting on tolerant rootstocks. Citron watermelon (*Citrullus lanatus* var. *citroides*) is well known for its high drought tolerance and has excellent potential as a watermelon rootstock. There is a high demand for novel citron watermelon cultivars with rootstock potential. Doubled haploid (DH) technology enables the breeding of genetically uniform and fully homozygous plants in a single generation. However, no research has been conducted on the haploidization of citron watermelon using the irradiated pollen technique. The current study aimed to determine the efficiency of gamma-ray sources (Cobalt: Co^{60} and Cesium: Cs^{137}), irradiation doses (200, 250, and 300 Gy of Co^{60}; 100, 150, 200, and 250 Gy of Cs^{137}), and genotypes (11 citron watermelon lines) on the production of pure citron lines via the irradiated pollen technique (parthenogenesis). The embryos were grown on MS medium supplemented with 0.4 mg/L indole-3-butyric acid. Stomatal observations and flow cytometry were used to conduct ploidy studies on 3–4 weeks old parthenogenic plantlets. The Co^{60} treatments yielded eight haploid and two mixoploid plants, while the Cs^{137} treatments yielded 14 haploid and one mixoploid. These findings demonstrated that gamma rays from both radiation sources were effective in parthenogenic embryo induction, and Cs^{137} was discovered to be a reliable alternative to conventional Co^{60}.

Key message

The efficiency of gamma-ray sources, irradiation doses, and genotypes were investigated. The parthenogenic embryo induction, and Cs137 was discovered to be a reliable alternative to conventional Co60.

Keywords Rootstock · Citron watermelon · Irradiation sources · Haploidization

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Introductin

Sweet watermelon (*Citrullus lanatus* var. *lanatus*) belongs to the Citrullus genus in the Cucurbitaceae family. It ranks second in global vegetable production behind tomatoes and is cultivated in field and greenhouse environments. China is the world leader in production, with 61.5 million tons, followed by Turkey at 3.9 million tons (FAO 2022). Consumed as a vegetable, it contains a high water content and many nutritional properties (sugar, antioxidants, and bioactive phytochemicals). Its seeds also serve as appetizers (Seymen et al. 2021).

Global climate change is expected to considerably impact agricultural production. It is now widely acknowledged that there will be challenges in the future in providing for the world population's food and nutritional demands, necessitating immediate attention. Drought is the most significant abiotic stressor resulting from climate change, threatening agricultural production due to its ability to disrupt rainfall patterns and the hydrological cycle (Yavuz et al. 2023). Sweet watermelon requires adequate water for optimal productivity, and poor irrigation significantly reduces yield and quality (Malambane et al. 2021). In such instances, deficit irrigation and drought-tolerant rootstocks provide critical strategies for mitigating and overcoming drought stress (Kurtar et al. 2024). Given that grafted seedlings are used for most conventional watermelon production, the current watermelon seed industry primarily focuses on developing F1 hybrid varieties in the globe. Thus breeding of F1 hybrid rootstocks with desirable agricultural characteristics has also become a significant concern. The drought tolerance levels or tolerance capacities of the rootstocks currently used in watermelon cultivation are still not at the desired level, and commercial watermelon rootstocks (commonly produced from *Cucurbita maxima x Cucurbita moschata* and small amounts of *Lagenaria*) only partially tolerate drought stress (Babaoglu and Türkmen 2017). Citron watermelon (*Citrullus lanatus* var. *citroides*), a drought-tolerant species within the Citrullus genus closely related to sweet watermelon (Mandizvo et al. 2022), has a high breeding potential for watermelon cultivation in arid and semi-arid areas (Seymen et al. 2021; Yavuz et al. 2023; Kurtar et al. 2024). Citron watermelon is also resistant to root-knot nematode (*Meloidogyne incognita*) (Thies et al. 2015) and Fusarium wilt race 2 (Wechter et al. 2012). Thus, it can be employed as a rootstock in watermelon to combat both biotic and abiotic stress. Its shorter vegetation period (90–120 days) than winter squash and pumpkin (150–180 days) allows for seed harvesting twice a year.

The preliminary step in F1 hybrid breeding is obtaining parental lines with high homozygosity, an essential factor for increasing heterosis. In classical breeding methods, achieving the desired homozygosity level requires 6–8 generations of inbreeding (selfing), especially in highly open-pollinated species such as watermelon. Tissue culture techniques known as gametic embryogenesis (in vitro techniques like androgenesis and gynogenesis and in situ techniques like parthenogenesis) provide significant convenience to breeders within the scope of accelerated breeding techniques to improve new F1 hybrids (Metin et al. 2024). Doubled haploid (DH) technology can produce completely homozygous lines in a single generation. In addition, reducing the size of the initial population to get the targeted genotype with the desired agronomic traits improves breeding efficiency (Kurtar et al. 2020). Thus, while significant time, labor, and cost savings are achieved, constantly introducing new varieties to the market is also accelerated (Kurtar and Balkaya 2010).

Parthenogenesis (irradiated pollen technique) is the primary DH technique utilized in haploidization in the Cucurbitaceae family. The approach works by irradiating anthers (one day before anthesis) with mature pollen mother cells from various sources (commonly Co^{60}-induced gamma-ray). The primary goal of irradiation is to produce genetically inactive pollen (with eliminated generative cells) capable of germination on the stigma while also delivering parthenogenic stimulation to maternal haploid embryos (Kurtar and Balkaya 2010).

Parthenogenesis using the irradiated pollen technique has been successful in the Cucurbitaceae family in *Citrullus lanatus* var. *lanatus* (Gürsöz et al. 1991; Sari 1994; Sari et al. 1994; Taşkın et al. 2013; Sari and Solmaz 2021), *Cucumis melo* L. (Lotfi et al. 2003), *Cucumis sativus* L. (Lotfi et al. 1999; Dolcet-Sanjuan et al. 2006; Salehian et al. 2023; Parkash et al. 2024), *Cucurbita pepo* L. (Kurtar et al. 2002, 2021; Berber 2009; Baktemur et al. 2014; Kurtar and Seymen 2021; Yapıcı et al. 2023), *Cucurbita moschata* Duch. (Kurtar et al. 2009), *Cucurbita maxima* Duch. (Kurtar and Balkaya 2010), *Lagenaria siceraria* (Malign) Stanley (Guler et al. 2017), *Benincasa hispida* (Thunb.) Cogn. (Liu et al. 2024), *Momordica charantia* L. (Reshmika et al. 2024), and *Cucumis melo* var. *flexuosus* (Taner et al. 2000). In addition, the irradiated pollen technique is actively used to obtain pure lines for the production of F1 hybrid varieties in melon (Sari et al. 2009; Göçmen et al. 2017; Şimşek et al. 2023). Irradiated pollen can also stimulate parthenogenic embryos and produce haploid plants in cucumber and squash, which can be integrated into breeding programs (Yapıcı et al. 2023).

While several published studies have documented the successful application of the parthenogenesis technique in sweet watermelon, there is currently no evidence of its successful implementation in citron watermelon. Furthermore, some factors, such as genotype, irradiation sources, irradiation doses, the composition of the induction media, culture conditions, embryo development stages, growing season, and donor conditions, limit the effectiveness of haploidy frequency using the irradiated pollen technique (Kurtar et al. 2020).

The current study aimed to determine the efficacy of various gamma-ray sources and irradiation doses in producing in vitro haploid plants from in situ-induced haploid embryos in prospective citroides rootstock candidates. To the author's knowledge, this is the first report of the successful production of citron watermelon using the parthenogenesis technique.

Materials and methods

Plant materials, experimental area, and crop management practices

Eleven morphologically different citron watermelon elite lines (CW4, CW6, CW8, CW9, CW11, CW13, CW14, CW15, CW16, CW17, and CW21) originating from Turkmenistan at the S3 filial (at the self-pollination 3rd stage) generation were used as donors. The current study was conducted in the greenhouse utilizing a growth chamber at the plant tissue culture laboratory of Selcuk University's Agriculture Faculty in 2022 and 2023. The study area spans 32° 30' E longitudes and 38° 02' N latitudes, with an average elevation of 1105 m above sea level.

Seeds of the citron watermelon (CW) lines were sown in plastic vials (cell volume of 140 cm^3 and 32 cells per vial) containing peat moss on March 2, 2022, and March 4, 2023, using Co^{60} and Cs^{137}, respectively. Meanwhile, 40 kg/ha N, 50 kg/ha K, and 35 kg/ha P were applied during soil preparation based on the soil analysis carried out for both growing periods in the greenhouse. The planting rows were laid out and covered with black polyethylene plastic film. On March 22nd and 28th, 2023, 30 healthy and hardened seedlings from the line at the 3–4 true leaf stage were planted with a 60 × 100 cm spacing. The plants were trained to a single-stem nature, and 50 kg/ha N, 60 kg/ha K, and 35 kg/ha P were applied with quantity adjustments throughout the cultivation period using a drip-irrigation system. The plants were regularly pruned to promote larger inflorescences. Disease (*Erysiphe cichoracearum* and *Sphaerotheca fuliginea)* and pests (*Tetranychus urticae* Koch. and *Bemisia tabaci*) were managed with systemic fungicides and pesticides.

Preparing flower buds, irradiation and pollination

Male flower buds were retrieved from donors the day before anthesis in as many equal numbers as possible for the irradiation treatment. Female flowers were isolated directly in monoecious lines and after emasculation in andromonoic lines using cloth bags around noon. The male flower buds were placed in 90 × 15 mm plastic petri dishes and irradiated at 200, 250, and 300 Gy (Gy) for Co^{60} (as per Sari et al. 1994; Taşkın et al. 2013) on 11, 16, 20, 23, 27, and 30 May 2022. Since the Cs^{137} source was used for the first time on CW and the degree of its effect was unknown, a range of irradiation doses was tested, and 50, 100, 150, 200, and 250 Gy doses were administered on May 25 and 29, as well as June 1, 5, and 13, 2023. Irradiation experiments were conducted at various periods to evaluate the effects of irradiation duration on parthenogenic embryo induction at TENMAK (Turkish Energy, Nuclear and Mineral Research Agency) (Fig. 1).

Following irradiation, the buds' petal, sepal, and pedicels were excised, and only the anthers were incubated on filter papers placed in petri dishes at room temperature overnight to allow the pollen sacs to rupture and the pollen grains to be released. Irradiated pollens were used to pollinate newly opened and vigorous female flowers that had been isolated the day before anthesis using a soft-tipped brush in the morning of the following day, between 07:00 and 09:00 am. Pollination experiments employed "0" days (the day following irradiation) and one-day-old pollen. The irradiated pollens were stored in the refrigerator at +4 ^{0}C until use. Female flowers were then isolated using cloth bags to avoid external pollen contamination, labeled, and recorded (Fig. 2). The bags were removed three to four days after pollination.

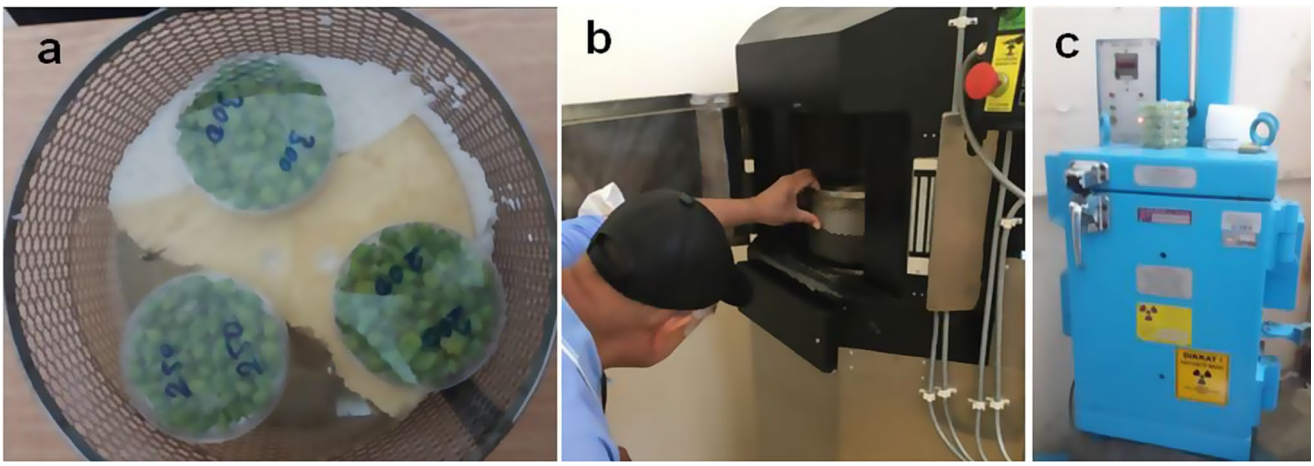

Fig. 1 Irradiation of male flower buds. **a** Male flower buds ready for irradiation. **b** Male flower buds irradiated with Co^{60}. **c** Male flower buds irradiated with Cs^{137}

Fig. 2 Application of irradiated pollen to female organs. **a** Anthers of irradiated flowers. **b** Pollination of female flowers with irradiated pollen grains. **c** Isolation of pollinated female flowers. **d** Flowers pollinated with irradiated pollen

Fig. 3 Seeds extracted from fruits at various stages of maturation

Fruit harvesting, extraction, and embryo culture

Immature fruits were harvested 21–24 days after pollination and stored in the storage chamber at 16 °C until extraction. The fruits were rinsed with running tap water for 15 min to remove gritty debris. They were immersed in a 20% (v/v) commercial bleach solution for 30 min for surface sterilization. Excess surface water from fruits was desiccated on filter paper and then flame-sterilized with 96% ethanol, followed by UV light for 15 min in a laminar flow sterile cabinet. Seeds were subsequently removed from the flesh and classified based on maturity stage (Fig. 3). When the seeds were first extracted from the fruits, they were slippery and had thick testa, making them difficult to handle and open. To help with this, they were left in a sterile cabinet for 30 min to dry partially. All seeds, excluding those with a transparent and soft structure, were extracted under axenic conditions using sterilized forceps and scalpels. The extraction process was the most intensive and time-consuming aspect of the investigation. Embryos were gently rescued and cultured in magenta boxes and culture tubes containing solid MS media (Murashige and Skoog 1962), supplemented with 0.4 mg/L IBA. The media was solidified with 8 g/L agar-agar, and the pH was adjusted to 5.8 for all processes. Embryos were incubated in a growth chamber at 26 ± 1 °C with white fluorescent 32 W lamps at a light intensity of 22 µmol/m^2/s under a 16 h/8 h day/night photoperiod (Fig. 4). Embryos were classified and documented based on their type and stage of development (Kurtar et al. 2002; Kurtar and Balkaya 2010).

Transplantation and acclimatization

Following 15–20 days of embryo culture, plantlets with healthy roots and shoots were transferred to the fresh MS medium for further development. Plants aged 21–35 days old that had completed their development were subjected to acclimatization. The process starts by opening the covers of magenta boxes and culture tubes for 5–7 days in a growth chamber at 26 ± 1 °C with a 16 h/8 h day/night photoperiod, 22 µmol/m^2/s light intensity, and 85% humidity. Plantlets were gently removed from culture vessels, and the roots were carefully rinsed under tap water. The plantlets were transplanted into plastic cups (150 cm^3) containing sterile peat moss (Klasman TS1). They were irrigated with water containing 0.2% fungicide solution (Maxim XL035FS) to prevent fungal infection at the start of acclimatization. The cups were subsequently covered with a transparent plastic bag and acclimatized in the same climatic conditions. The plastic bags were gradually opened and completely removed after 12 days (Fig. 5).

Ploidy level assessment

Plant ploidy levels were determined using stomatal observations (Kurtar and Balkaya 2010; Kurtar et al. 2020), flow cytometry (FCM) analysis, and morphological observations. The 3rd and 4th leaves of the shoot apex of acclimatized plants were examined to assess the number of chloroplasts on each side of the guard cells, stomata length

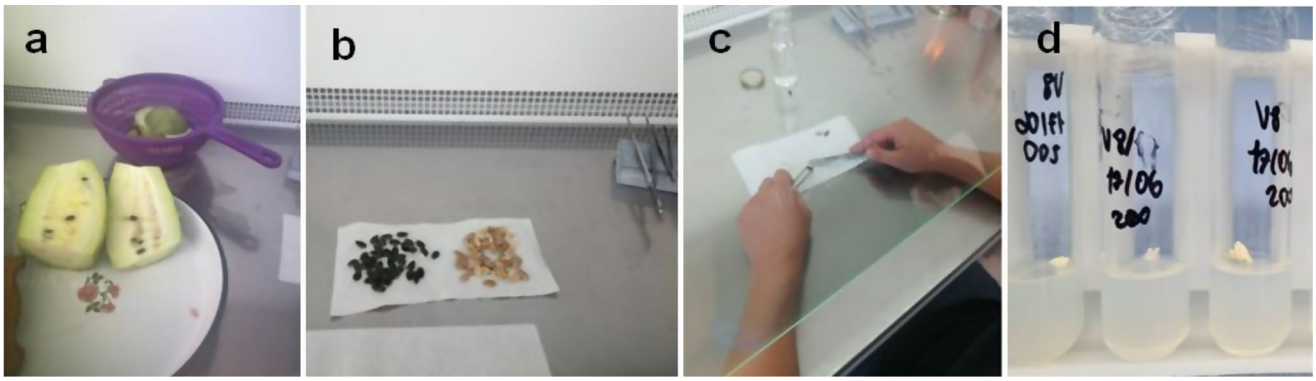

Fig. 4 Extraction and culturing of parthenogenic embryos. **a** Seeds extraction from fruits. **b** Seeds separation based on maturity. **c** Isolation of parthenogenic embryos. **d** Culturing parthenogenic embryos in regeneration media

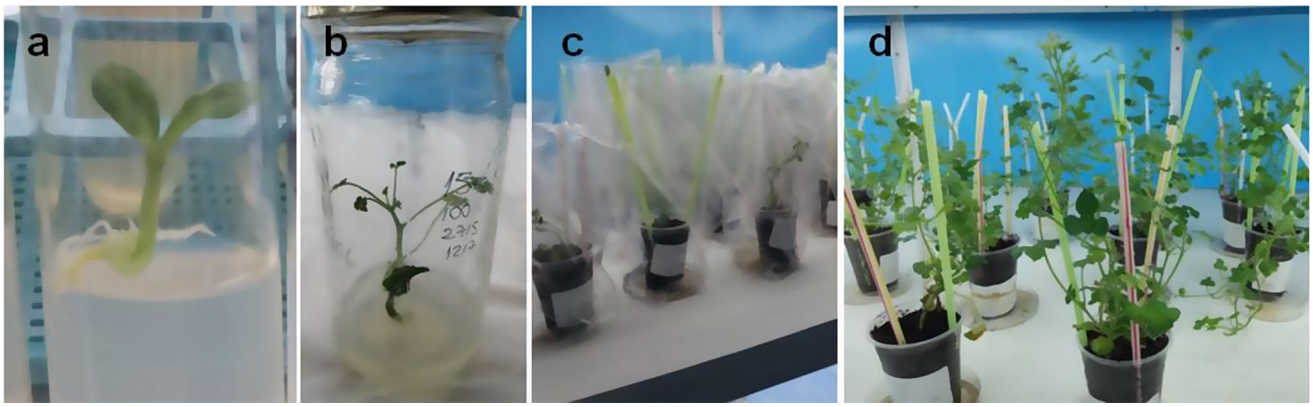

Fig. 5 Acclimatization of parthenogenic citron melon plants. **a** A young parthenogenic plantlet. **b** A parthenogenic plant with well-established roots and shoots. **c** Potted parthenogenic plants in a growth chamber. **d** Acclimatized parthenogenic plants in a growth chamber

and width (µm), and stomata density (stomata/mm^2). The lower epidermis of unwrinkled fresh leaves was first placed on a microscopic slide, and one drop of 1% $AgNO_3$ solution was added before being covered with a lamella and examined in three different areas (Kurtar and Seymen 2021).

FCM analysis was performed using nuclei samples isolated from the leaves of parthenogenic citron watermelon plants (Alan et al. 2021). Each sample contained approximately 20 mg of fresh citron melon leaf tissue and 30 mg of leaf tissue from an internal control plant (young rape seedlings; 2.3 pg/2 C DNA). Citron melon and rapeseed leaves were placed in 65 mm × 10 mm Petri dishes containing 1.5 mL ice-cold nuclei isolation buffer and sliced into strips using a scalpel. Nuclei samples were filtered through a 40-µm mesh, stained with propidium iodide, and placed in an ice bucket until analyzed using a Cell Lab Quanta SC flow cytometer (Beckman-Coulter). Over 3000 nuclei were analyzed in each sample. The DNA content of their nuclei determined the ploidy levels of parthenogenic citron melon plants compared to the standards. Plant development status, pollen production in male flowers, and flower sizes were also investigated as morphological observations.

Data collection and statistical analysis

Fruit number (FN), seed number (SN), mean seed number (MSN), embryo number (EN), mean embryo number (MEN), embryonic development (ED), plant number (PN), ploidy status (PS), and haploidy frequency in 100 embryos (HF; %) were evaluated for genotypes (G) and irradiation doses (D; Gray). Due to unequal test materials, the results of the evaluated parameters are only presented as percentages (%).

Results

Co^{60} experiments

Effects of irradiation doses and genotypes on seed formation and embryo induction

The genotypes and irradiation doses influenced seed and embryo formation in CW (Table 1). The extraction of 271 fruits resulted in the dissection of 5112 seeds at various maturity stages and the rescue and culture of 151 embryos

Table 1 Fruit number (FN), seed number (SN), mean seed number (MSN), embryo number (EN), mean embryo number (MEN), embryo shape (ES), plant number (PN), ploidy status (PL), and haploidy frequency (HF) in 100 embryos (%) across genotypes (G) and irradiation doses (D; Gray)

G	D	FN	SN	MSN	EN	MEN	ED	PN	PS	HF
Genotype and Irradiation dose										
4	**200**	11	328	29.8	4	0.36	C	2	D	0
	250	14	241	17.2	5	0.36	4 C, 1HE	4	3D, 1 H	20.0
	300	14	127	9.1	1	0.07	G	0	0	0
	∑/M	***39***	***696***	***17.8***	***10***	***0.26***	***8 C*, *1HE*, *1G***	***6***	***5D*, *1 H***	***10.0***
6	**200**	7	287	41.0	4	0.57	C	4	D	0
	250	11	209	19.0	4	0.36	3 C, 1HE	3	2D, 1 H	25.0
	300	8	161	20.1	9	1.13	7 C, 1HE, 1G	7	6D, 1 H	11.1
	∑/M	***26***	***657***	***25.3***	***17***	***0.65***	***14 C*, *2HE*, *1G***	***14***	***12D*, *2 H***	***11.8***
8	**200**	8	194	24.3	1	0.13	C	0	0	0
	250	9	151	16.8	1	0.11	C	0	0	0
	300	9	139	15.4	0	0	0	0	0	0
	∑/M	***26***	***484***	***18.6***	***2***	***0.08***	***2 C***	***0***	***0***	***0***
9	**200**	10	202	20.2	11	1.1	C	8	D	0
	250	5	91	18.2	4	0.8	3 C, 1HE	4	3D, 1 H	25.0
	300	8	72	9.0	1	0.13	C	0	0	0
	∑/M	***23***	***365***	***15.9***	***16***	***0.70***	***15 C*, *1HE***	***12***	***11D*, *1 H***	***6.3***
11	**200**	7	306	43.7	2	0.29	C	1	D	0
	250	5	167	33.4	1	0.20	C	0	0	0
	300	8	48	6.0	0	0	0	0	0	0
	∑/Avr	***20***	***521***	***26.1***	***3***	***0.15***	***3 C***	***1***	***D***	***0***
13	**200**	14	226	16.1	6	0.43	C	4	D	0
	250	8	103	12.9	2	0.25	C	2	1D, 1 M	0
	300	7	77	11.0	0	0	0	0	0	0
	∑/M	***29***	***406***	***14.0***	***8***	***0.28***	***8 C***	***6***	***5D*, *1 M***	***0***
14	**200**	5	184	36.8	2	0.40	C	1	D	0
	250	7	57	8.1	2	0.29	C	1	D	0
	300	8	41	5.1	0	0	0	0	0	0
	∑/M	***20***	***282***	***14.1***	***4***	***0.20***	***4 C***	***2***	***D***	***0***
15	**200**	11	251	22.8	4	0.36	3 C, 1HE	4	3D, 1 H	25.0
	250	7	122	17.4	6	0.86	C	4	3D, 1 H	16.7
	300	5	59	11.8	1	0.20	C	1	D	0
	∑/M	***23***	***432***	***18.8***	***11***	***0.48***	***10 C*, *1HE***	***9***	***7D*, *2 H***	***18.2***
16	**200**	10	237	23.7	4	0.40	C	3	2D, 1 M	0
	250	5	104	20.8	1	0.20	C	0	0	0
	300	8	109	13.6	0	0	0	0	0	0
	∑/M	***23***	***450***	***19.6***	***5***	***0.22***	***5 C***	***3***	***2D*, *1 M***	***0***
17	**200**	8	173	21.6	18	2.25	18 C	12	D	0
	250	7	107	15.3	6	0.86	C	2	D	0
	300	9	91	10.1	4	0.44	2 C, 1HE, 1G	2	1D, 1 H	33.3
	∑/M	***24***	***371***	***15.5***	***28***	***1.17***	***27 C*, *1HE*, *1G***	***16***	***15D*, *1 H***	***3.6***
21	**200**	10	307	30.7	44	4.40	44 C	33	D	0
	250	3	78	26.0	2	0.67	1G	1	D	0
	300	5	63	12.6	1	0.20	1G	1	H	100.0
	∑/M	***18***	***448***	***24.9***	***47***	***2.61***	***44 C*, *2G***	***35***	***34D*, *1 H***	***2.1***
	Irradiation dose									
	200	101	2695	26.7	106	1.05	102 C, 1HE	64	62D, 1 H, 1 M	0.94
	250	81	1430	17.7	30	0.37	27 C, 3HE, 1G	29	24D, 4 H, 1 M	13.33
	300	89	987	11.1	15	0.17	12 C, 2HE, 3G	11	8D, 3 H	20.00
	Overall	***271***	***5112***	***18.86***	***151***	***0.56***	***141 C*, *6HE*, *4G***	***104***	***94D*, *8 H*, *2 M***	***5.29***

C: Cotyledon; **HE**: Heart; **G**: Globular; **H**: Haploid, **M**: Mixoploid, **D**: Diploid

on modified MS media. However, a linear relationship between the increase in radiation doses and the number of fruits could not be established. The number of fruits decreased relatively at 250 and 300 Gy radiation doses. Among the lines, CW4 performed the best with 39 fruits, followed by CW13 with 29 fruits. CW11 showed the lowest performance, with only 20 fruits.

The highest total seed number (SN) was extracted from CW4 (696) and CW6 (657), whereas CW14 (282), CW9 (365), and CW17 (371) generated the lowest SN values. The mean number of seeds (MSN) per fruit was 18.86, while the average number of embryos per fruit was 0.56. The MSN per fruit varied between genotypes, ranging from 14.0 (CW13) to 26.1 (CW11), with a mean number of embryos (MEN) of 0.04 in CW8 and 2.61 in CW21.

In general, reductions in MSN and MEN values were found to occur concurrently with an increase in irradiation doses when both genotypes and radiation doses were analyzed. The MSN per fruit ranged from 5.1 (CW14 and 300 Gy) to 43.7 (CW11 and 200 Gy). In contrast, pollination with a 300 Gy dose did not produce parthenogenic embryos (EN) in CW8, CW11, CW13, CW14, and CW16. Although the seeds formed a well-developed testa, they were empty and lacked any embryonal structure, making cultivating embryos from some lines impossible.

The MEN per fruit varied between 0.07 (CW4 and 300 Gy) and 4.40 (CW21 and 200 Gy), depending on the combinations from which embryos were produced. The highest MEN was obtained from CW21 and 200 Gy at 4.40, followed by CW17 and 200 Gy at 2.25. CW21 (47 embryos) and CW21 (28 embryos) were determined as prominent lines in terms of total embryo number (EN). Furthermore, CW8, CW11, and CW14 yielded relatively reduced embryo yields of 2, 3, and 4 embryos, respectively. When only the irradiation doses were considered, the highest number of fruits was 101 at 200 Gy, followed by 81 at 250 Gy, and 89 fruits at 300 Gy. Similarly, the 200 Gy dose produced 2695 seeds and 106 embryos, while the 300 Gy dose yielded 987 seeds and 15 embryos.

Effects of irradiation doses and genotypes on embryo type, plant number, ploidy status, and haploidy frequency

The bulk of embryos retrieved from seeds were in the cotyledon (C) stage, with only a few having heart (HE) and globular (G) stages (Fig. 6). Overall, 141 C (93.38%), 6 HE (3.97%), and 4 G (2.65%) embryos were cultured for the investigated genotypes (Table 1). One hundred four cultured embryos were successfully regenerated into whole plants and acclimatized, with a success rate of 68.87%. In terms of the number of embryos cultured, CW21 (35 plants) had the highest average number of plants (PN), followed by CW17 (16 plants) and CW6 (14 plants). The lowest plant regeneration rate was obtained for CW11, CW14, and CW16, with 1, 2, and 3 plants, respectively. Furthermore, regeneration into a plant was not achieved with CW8, and no plants could be obtained.

C embryos were predominant (96.2%) in all genotypes at 200 Gy treatment, 90% at 250 Gy, and 80% at 300 Gy. Considering the combinations from which plants were obtained, the PN ranged from 1 (CW11) to 33 (CW21) at 200 Gy. At 250 Gy, PN varied between 1 (CW14 and CW21) and 4 (CW4, CW9, and CW15), whereas at 300 Gy, it was between 1 (CW15 and CW21) and 7 (CW6).

Irradiation doses and embryo type have been shown to affect plant ploidy status (PS) and haploidy frequency (HF). Haploid plants could not be obtained from the CW8, CW11, CW13, CW14, and CW16 lines; however, CW6 and CW16 produced two haploid plants each. The mean HF in the lines that produced haploid plants ranged from 2.1 (CW21) to 18.2 (CW15). Most haploid plants developed from HE and G embryos, whereas C embryos that covered the entire seed could not produce haploid plants. The 200 Gy treatment resulted in predominantly C embryos and only one haploid plant, yielding a low HF (0.94). In contrast, a relatively high proportion of HE and G embryos at 250 Gy and 300 Gy doses gave rise to four and three haploid plants, respectively with HF values of 13.33 and 20.00. In addition to haploid (n) and diploid (2n) plants, two mixoploid ($n+2n$) plants were detected at 200 Gy (CW16) and 250 Gy (CW13) doses. Overall, 94 diploid, eight haploid, and two mixoploid plants were obtained from 151 cultured embryos, with an HF of 5.29.

Cs^{137} experiments

Effects of irradiation doses and genotypes on seed formation and embryo induction

In Cs^{137} studies, genotypes and irradiation doses substantially affected seed and embryo formation in the CW lines, similar to Co^{60} (Table 2). One hundred twenty-six fruits were extracted, 6120 seeds at various maturity stages were dissected, and 630 embryos were rescued and cultured on modified MS media. There was no linear relationship between radiation doses and fruit number (FN); nonetheless, there were variances in FN between the lines. Although CW9 (28 fruits) and CW13 (22 fruits) yielded the highest FN, CW16 (6 fruits) had the lowest performance. CW13 (1217 seeds) produced the highest total seed number (SN), followed by CW9 (1166 seeds). CW8 (198 seeds) and CW16 (220 seeds) had the lowest SN. The mean seed number (MSN) per fruit was 48.57, while the number of embryos per fruit was 5.00. While the MSN ranged from 24.8 (CW8) to 74.3

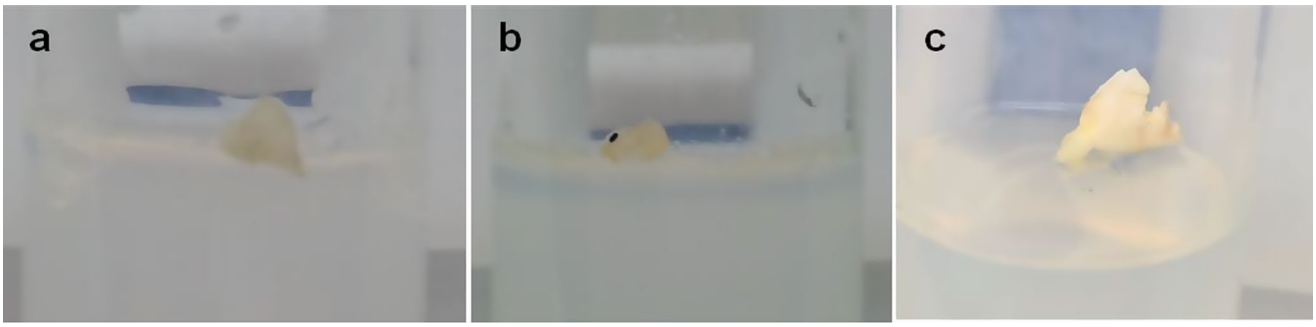

Fig. 6 Heart (**a**), globular (**b**) and cotyledone (**c**) embryos in culture conditions

(CW11), mature seeds and parthenogenic embryos (EN) were not observed in CW8 and CW16 after pollination with 250 Gy dose, and the fruits were parthenocarpic (Fig. 7). A combination of several lines and irradiation doses (CW8 and CW17 at 200, CW11 at 200 and 250 Gy, and CW13 and CW16 at 250 Gy) generated seeds; however, the seeds were empty, preventing embryo culture. The mean EN values varied greatly, with the lowest at 19 in CW17 and the highest at 159 in CW9 (Table 2).

Increased irradiation doses resulted in declines in MSN, EN, and MEN values across genotypes. In seed-containing combinations, MSN per fruit ranged from 7.0 (CW6 and CW14 at 250 Gy) to 110.8 (CW13 at 100 Gy). The mean embryo number (MEN) varied from 1.63 in CW8 to 6.62 in CW13. Among the combinations from which embryos were retrieved, CW13 at 100 Gy had the highest MEN per fruit at 24.0 (96 embryos), while CW4 at 150 and 200 Gy had the lowest MEN at 0.07 (2 embryos). Furthermore, CW11 (17.5) and CW6 (14.00) were identified as prominent lines based on MEN. Across the irradiation doses tested, 100 and 150 Gy doses produced relatively higher FN. FN was 37 at 100 Gy, 38 at 150 Gy, 31 at 200 Gy, and 30 at 250 Gy. The 100 Gy dose produced 2935 seeds and 424 embryos, while the 250 Gy dose yielded 516 seeds and 330 embryos.

Effects of irradiation doses and genotypes on embryo type, plant number, ploidy status, and haploidy frequency

The bulk of embryos extracted from the seeds were in the cotyledon (C), with very few in the heart (HE) or globular (G). Overall, 604 C (95.87%), 9 HE (1.43%), and 17 G (2.70%) embryos were cultured for the genotypes under investigation (Table 1). Three hundred sixty-four embryos were rescued, successfully regenerated into a whole plant, and acclimatized, with a conversion rate of 57.78%. Transformation of the embryo into a plant was achieved for all lines. In terms of embryos cultured, CW9 and CW13 (95 plants) had the highest average PN, followed by CW21 (40 plants) and CW15 (27 plants). CW8, CW16, and CW17 produced the lowest PN with 8, 9, and 11 plants, respectively.

At 100 Gy, all genotypes produced C embryos, while at 150, 200, and 250 Gy, the percentages were 98.28%, 76.67%, and 66.67%, respectively. Considering the combinations from which plants were obtained, the PN ranged from 4 (CW16) to 74 (CW13) at 100 Gy. PN varied between 1 (CW4, CW8, and CW17) and 19 (CW13) at 150 Gy, 1 (CW4, CW14, CW15, and CW16) and 9 (CW9) at 200 Gy, and 1 (CW4, and CW14) and 4 (CW21) at 250 Gy.

Irradiation doses and embryo types also affected plant ploidy status (PS) and haploidy frequency (HF). Except for CW8 and CW11, all lines produced haploid plants. The mean HF in the lines that produced haploid plants ranged from 0.7 (CW13) to 7.1 (CW6). HE and G embryos produced the bulk of haploid plants, while C embryos were rarely fertile. While no haploid plants could be obtained from the 100 and 150 Gy treatments, 5 and 9 haploid plants were obtained from the 200 and 250 Gy doses, with HF values of 8.33 and 30.00, respectively. In addition to haploid (n) and diploid (2n) plants, one mixoploid ($n+2n$) plant was determined at 200 Gy (CW9). Overall, 349 diploid, 14 haploid, and one mixoploid plant were produced from 630 cultured embryos, with an HF of 2.22.

Ploidy status of plants

Diploid plants possessed 10 or 12 chloroplasts in guard cells, while haploid plants had 6 or 8 chloroplasts, and both types of stomata were observed in mixoploid plants (Fig. 8). The average chloroplast count was 11.42 in diploids, 6.64 in haploids, and 90.2 in mixoploids. The average stomata length and width were 25.27 and 19.76 µm in diploids, 16.49 and 13.40 µm in haploids, and 21.81 and 17.79 in mixoploids. While diploid plants had larger stomata than haploids and mixoploids, haploid plants had a higher stomata density (597.3 stoma/mm^2) than mixoploids (527.7 stoma/mm^2) or diploids (378.4). The peaks of haploid and control diploid plants differed in the FCM histogram. The haploid and control diploid plants' average genome sizes (pg) were determined as 0.44 and 0.91, respectively (Fig. 9).

Table 2 Fruit number (FN), seed number (SN), mean seed number (MSN), embryo number (EN), mean embryo number (MEN), embryo shape (ES), plant number (PN), ploidy status (PS), haploidy frequency (HF) in 100 embryos (%) across genotypes (G) and irradiation doses (D; Gray)

G	D	FN	SN	MSN	EN	MEN	ES	PN	PS	HF
Genotype and Irradiation dose										
4	**100**	4	256	64.0	50	12.50	C	18	D	0
	150	3	191	63.7	2	0.67	C	1	D	0
	200	3	108	36.0	2	0.67	1 C, 1G	1	D	0
	250	1	8	8.0	3	3.00	1 C, 1HE, 1G	1	H	33.3
	∑/M	***11***	***563***	***53.0***	***57***	***5.18***	***54 C*, *1HE*, *2G***	***21***	***20D*, *1 H***	***1.8***
6	**100**	1	103	103.0	14	14.00	C	10	D	0
	150	2	145	72.5	6	3.00	C	3	D	0
	200	2	62	31.0	5	2.50	3 C, 1HE, 1G	2	1D, 1 H	20.0
	250	3	21	7.0	3	1.00	1 C, 2G	2	1D, 1 H	33.3
	∑/M	***8***	***351***	***43.9***	***28***	***3.50***	***24 C*, *1HE*, *3G***	***17***	***15D*, *2 H***	***7.1***
8	**100**	2	162	81.0	11	5.50	C	7	D	0
	150	2	22	11.0	2	1.00	C	1	D	0
	200	2	14	7.0	0	0	0	0	0	0
	250	2	0	0.0	0	0	0	0	0	0
	∑/M	***8***	***198***	***24.8***	***13***	***1.63***	***13 C***	***8***	***8D***	***0.0***
9	**100**	10	593	59.3	96	9.60	C	71	D	0
	150	4	212	53.0	24	6.00	C	11	D	0
	200	7	204	29.1	27	3.86	21 C, 2HE, 4G	9	7D, 1 H, 1 M	3.7
	250	7	157	22.4	12	1.71	10 C, 2G	4	2D, 2 H	16.7
	∑/M	***28***	***1166***	***42.6***	***159***	***5.68***	***151 C*, *2HE*, *6G***	***95***	***91D*, *3 H*, *1 M***	***1.9***
11	**100**	2	292	146.0	35	17.50	C	21	D	0
	150	3	207	69.0	14	4.67	C	7	D	0
	200	2	80	40.0	0	0.00	0	0	0	0
	250	1	15	15.0	0	0.00	0	0	0	0
	∑/M	***8***	***594***	***74.3***	***49***	***6.13***	***49 C***	***28***	***28D***	***0***
13	**100**	4	443	110.8	96	24.00	C	74	D	0
	150	5	370	74.0	37	7.40	C	19	D	0
	200	6	262	43.7	6	1.00	5 C, 1HE	2	1D, 1 H	16.7
	250	6	142	23.7	0	0	0	0	0	0
	∑/M	***21***	***1217***	***58.0***	***139***	***6.62***	***138 C*, *1HE***	***95***	***94D*, *1 H***	***0.7***
14	**100**	3	247	82.3	15	5.00	C	9	D	0
	150	1	22	22.0	4	4.00	C	2	D	0
	200	2	18	9.0	3	1.50	2 C, 1G	1	D	0
	250	2	14	7.0	4	2.00	2 C, 1HE, 1G	1	H	25.0
	∑/M	***8***	***301***	***38.9***	***26***	***3.25***	***23 C*, *1HE*, *2G***	***13***	***12D*, *1 H***	***3.8***
15	**100**	3	271	90.3	40	13.33	C	21	D	0
	150	3	212	70.7	8	2.67	C	3	D	0
	200	2	84	42.0	5	2.50	C	1	D	0
	250	3	72	24.0	4	1.33	3 C, 1HE	2	1D, 1 H	25.0
	∑/M	***11***	***639***	***58.1***	***57***	***5.18***	***56 C*, *1HE***	***27***	***26D*, *1 H***	***1.8***
16	**100**	2	150	75.0	12	6.00	C	4	D	0
	150	1	49	49.0	5	5.00	3 C, 1HE, 1G	4	D	0
	200	2	21	10.5	3	1.50	2 C, 1G	1	H	33.3
	250	1	0	0.0	0	0	0	0	0	0
	∑/M	***6***	***220***	***36.7***	***20***	***3.33***	***17 C*, *1HE*, *2G***	***9***	***8D*, *1 H*, *1 M***	***5.0***
17	**100**	2	184	92.0	13	6.50	C	9	D	0
	150	2	161	80.5	4	2.00	C	1	D	0
	200	1	76	76.0	2	2.00	C, 1G	1	H	50.0
	250	2	49	24.5	0	0	0	0	0	0
	∑/M	***7***	***470***	***67.1***	***19***	***2.71***	***18 C*, *1G***	***11***	***10D*, *1 H***	***5.3***

Table 2 (continued)

G	D		FN	SN	MSN	EN	MEN	ES	PN	PS	HF
21	**100**	4		234	58.5	42	10.50	C	29	D	0
	150	2		73	36.5	10	5.00	C	3	D	0
	200	2		56	28.0	7	3.50	6 C, 1G	4	3D, 1 H	14.3
	250	2		38	19.0	4	2.00	3 C, 1HE	4	2D, 2 H	50.0
	∑/Avr	***10***		***401***	***40.1***	***63***	***6.30***	***61 C, 1HE, 1G***	***40***	***37D, 3 H***	***4.8***
	Irradiation dose										
	100	37		2935	79.32	424	11.46	424 C	275	D	0
	150	28		1664	59.43	116	4.39	114 C, 1HE, 1G	53	52D, 1 M	0
	200	31		985	31.77	60	1.94	46 C,4HE, 10G	21	16D, 5 H	8.33
	250	30		516	17.20	30	1.30	20 C, 4HE, 6G	15	6D, 9 H	30.00
	Overall	**126**		**6120**	***48.57***	***630***	***5.00***	***604 C, 9HE, 17G***	***364***	***349D, 14 H, 1 M***	***2.22***

C: Cotyledon; **HE**: Heart; **G**: Globular; **H**: Haploid, **M**: Mixoploid, **D**: Diploid

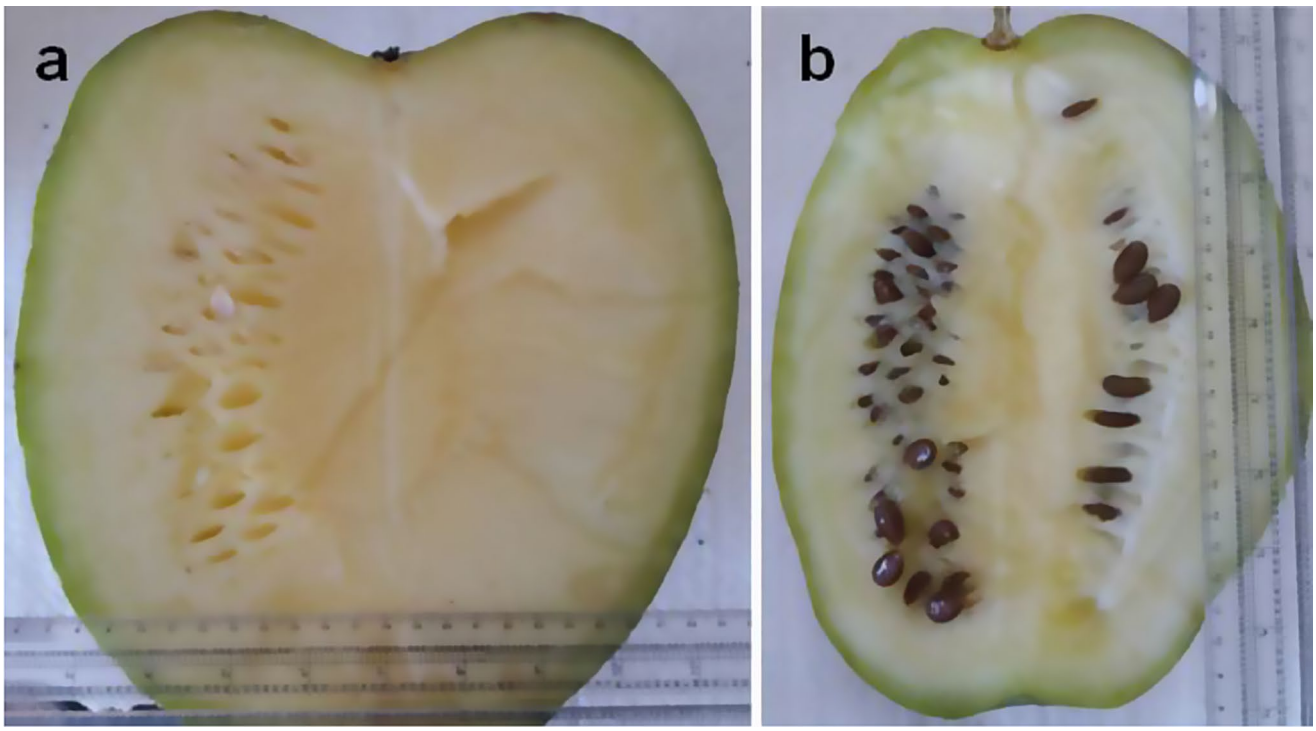

Fig. 7 Parthenocarpic fruits with immature (white and primarily transparent) seeds (a) and a fruit containing mature seeds at 200 Gy irradiation dose (b)

Morphological observations

Plants determined to be haploid via stomatal observations and flow cytometry were grown in greenhouse settings and morphologically examined in contrast to diploid plants. Morphologically, the sizes of the leaves and flowers, as well as the presence of pollen in male flowers, were investigated. The male flowers of haploid plants did not release pollen grains, and their leaves and flowers were smaller than those of diploid plants (Figs. 10 and 11).

Discussion

CW is a unique material for generating drought-tolerant rootstocks that ensure sweet watermelon yield and fruit quality while using less water, particularly in water-scarce regions (Seymen et al. 2021; Mandizvo et al. 2022; Yavuz et al. 2023; Kurtar et al. 2024). This study aims to investigate the efficacy of the dihaploidization process using the irradiated pollen technique for producing pure lines using CW lines selected from our gene pool. However, the irradiated pollen technique has only been the subject of a few studies in sweet watermelon (Gürsöz et al. 1991; Sari 1994; Sari et al. 1994; Taşkın et al. 2013; Sari and Solmaz 2021), with

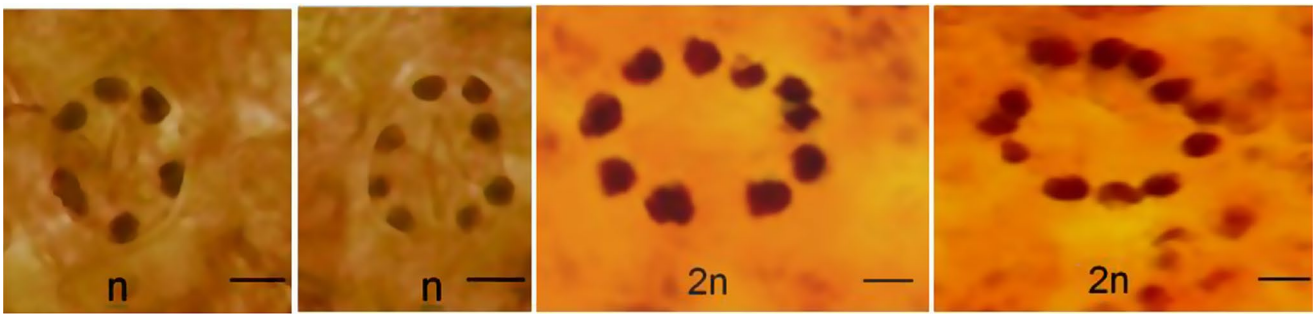

Fig. 8 Chloroplast count in haploid (n) and diploid (2n) plants (Bar: 5 µ)

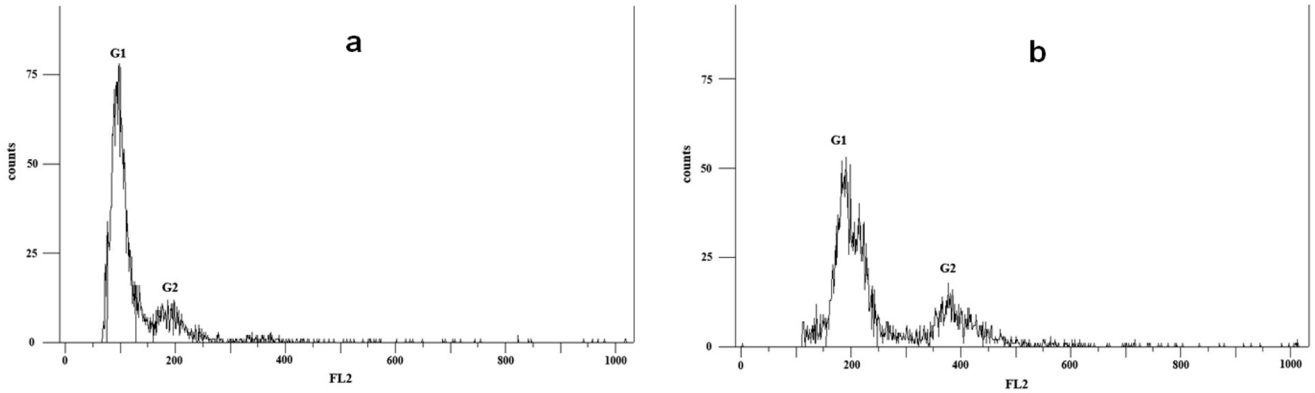

Fig. 9 Flow cytometry analysis of CW materials. **a** Parthenogenic haploid (n) plant. **b** Diploid (2n) donor plant grown from seed

no research on CW. These studies used various gamma-ray sources and irradiation doses to generate haploid plantlets. Nevertheless, a protocol for producing haploid plants successfully using the irradiated pollen technique is yet to be published, and some bottlenecks in the widespread utilization of this technique for generating F1 hybrid watermelon cultivars and rootstocks include genotype dependence, low proliferation, and plant regeneration potential.

We achieved embryo induction using both Co^{60} and Cs^{137}, albeit at different gamma-ray doses. It was determined that the 200 Gy radiation dose caused an inbreeding effect because almost all of the recovered plants' ploidy levels were diploid, even though the highest number of fruits and embryos in Co^{60} were rescued at that dose. Cs^{137} showed similar outcomes at 100 and 150 Gy doses. The high rate of diploid plants at low doses can be attributed to the incomplete inactivation of the generative nucleus in pollen, which fertilizes the egg cell and forms diploid embryos (Zhao et al. 2023).

Irradiation sources and doses significantly altered plant ploidy status (PS) and haploidy frequency (HF). All irradiation doses could produce haploid plants in Co^{60}, with the best results obtained after a 300 Gy dose with 20% HF. Furthermore, only 200 and 250 Gy treatments resulted in haploid plants and haploid parthenogenesis was not observed at 100 and 150 Gy in Cs^{137}, with 250 Gy being the most significant dose with 30% HF. Earlier studies on haploid induction using the irradiated pollen technique with Co^{60} indicated that the optimal dose for induction parthenogenesis in Cucurbitacea ranges from 50 to 500 Gy. Embryos and haploid plants were successfully induced with 50 to 150 Gy in *Cucurbita pepo* L. (Kurtar et al. 2002, 2021; Berber 2009; Baktemur et al. 2014; Kurtar and Seymen 2021; Yapıcı et al. 2023), *Cucurbita moschata* Duch. (Kurtar et al. 2009), and *Cucurbita maxima* Duch. (Kurtar and Balkaya 2010). Similar results were observed with 90 Gy in *Momordica charantia* L. (Reshmika et al. 2024), 100 Gy in *Benincasa hispida* (Thunb.) Cogn. (Liu et al. 2024), 50 to 125 Gy in *Lagenaria siceraria* (Teppner 2004; Guler et al. 2017; Zhao et al. 2023), and 300–350 Gy in *Cucumis melo* var. *flexuosus* (Taner et al. 2000). 200 to 300 Gy induced haploid plants in *Cucumis melo* L. (Lotfi et al. 2003; Lim and Earle 2008; Gonzalo et al. 2011; Godbole and Murthy 2012), *Citrullus lanatus* var. lanatus (Gürsöz et al. 1991; Sari et al. 1994; Taşkın et al. 2013; Sari and Solmaz 2021), and *Cucumis sativus* L. (Lotfi et al. 1999; Dolcet-Sanjuan et al. 2006; Salehian et al. 2023). However, a few studies on some of these species found that the optimum dose varies. For example, the optimal irradiation dose for *Cucumis sativus* has been reported to be 100 Gy (Lotfi et al. 1999), 500 Gy (Dolcet-Sanjuan et al. 2006; Parkash et al. 2024),

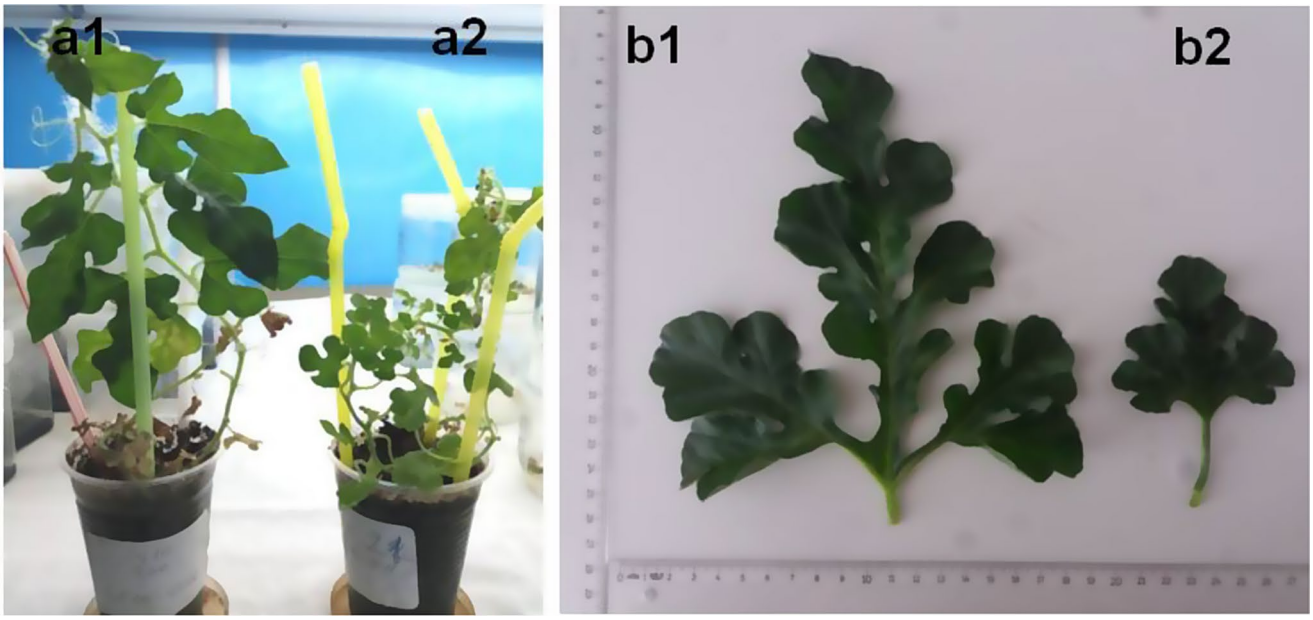

Fig. 10 The leaves diploid (a1 and b1) and haploid (a2 and b2) plants

Fig. 11 Diploid (a1 and b1) and haploid (a2 and b2) male flowers one day before and during anthesis, respectively

and 550 Gy (Bagheri et al. 2021), whereas it was 750 Gy for *Cucumis melo* (Sun et al. 2006).

In addition, varying degrees of success with in situ haploid induction with cobalt and difficulties accessing the cobalt source have caused researchers to employ alternative sources. Cs and X-rays have been used as irradiation sources in *Cucumis melo* (Lotfi et al. 2003; Dal et al. 2016) and *Cucurbita pepo* var. *styriaca* (Košmrlj et al. 2014). Cs^{137} could successfully induce haploid embryos in *Cucumis melo* with doses of 200 Gy (Dal et al. 2016) and 250 Gy (Lotfi et al. 2003). Furthermore, irradiation efficiency is determined by the radiation source (Goldschmidt et al. 1994), source activity, and dose rate (Kurtar and Balkaya 2010).

In our Co^{60} trials, 151 embryos from 5112 seeds were cultured. One hundred four of them regenerated into plants, with 94 being diploid (2n), eight being haploid (n), and two being mixoploid ($n+2n$). The HF for 100 seeds, 100 embryos, and 100 plants was 0.156, 5.30, and 7.69, respectively. In addition, in our Cs137 trials, 630 embryos were extracted from 6120 seeds, and 364 plants were generated, of which 14 were haploid, one was mixoploid, and the remaining plants were diploid. The HF for 100 seeds, 100 embryos, and 100 plants was 0.229, 2.22, and 3.85, respectively. Although Cs^{137}-induced irradiation produced more plants; the HF was higher in Co^{60}-induced gamma-ray irradiation. Co^{60} was more effective

and economical than Cs^{137} in terms of seed opening, embryo cultivation, plant regeneration, and ploidy determination. The three mixoploid plants (1 of Co^{60} and 2 of Cs^{137}) were evaluated as unique material since spontaneous doubling occurs frequently in mixoploid plants, and pure lines can be created without chromosome doubling experiments. Spontaneous doubling in in vitro haploid plants is widespread in some species, such as pepper and eggplant; however, there is no information on how this occurs in watermelon.

Gürsöz et al. (1991) revealed a haploidy frequency of 2.23 per 100 embryos, while Taşkın et al. (2013) reported 5.26 haploid embryos per 100 seeds in watermelon. Sarı et al. (1994) found that the number of embryos per 100 seeds varied across cultivars, ranging from 14.2% (Halep Karası) to 3.6 (Sugar Baby). Our findings were consistent with those of Gürsöz et al. (1991) but lower than those of Sari et al. (1994) and Taşkın et al. (2013). Although the HF in 100 plants and 100 embryos was relatively acceptable, very few embryos were obtained compared to the number of seeds extracted, resulting in low HF values in our study.

Genotypes affect HF just as much as radiation doses and sources, and CW lines react differently depending on the radiation source. In Co^{60}, the CW4, CW6, CW9, CW15, CW17, and CW21 lines generated haploid plants, while in Cs^{137}, all lines except CW8 and CW11 produced haploid plants. This demonstrated that employing multiple irradiation sources could boost the haploid induction rate, except for the recalcitrant lines such as CW8 and CW11, which did not yield haploid plants using either irradiation source. Previous studies (Kurtar and Balkaya 2010; Kurtar et al. 2020;) found that genotype, irradiation sources, irradiation doses, and embryo development stages restricted the HF in irradiated pollen technique.

Stomata size, density, chloroplast number, and FCM diagrams differed between haploid, diploid, and mixoploid plants. Stoma measurement and chloroplast counting proved more practical and cost-effective than FCM. These results indicated that stomata observations could successfully assess ploidy status in CW. Flow cytometry is a potentially helpful method for determining ploidy levels. However, it is expensive, labor-intensive, and requires specialized equipment. To estimate the ploidy level, morphological observation—which involves the diameters of the leaves and flowers and determining if pollen is present in male flowers—is an effective method in addition to stomatal observations and FCM, albeit time-consuming. Similar results were reported in watermelon (Sari et al. 1999), summer squash (Kurtar et al. 2002), pumpkin (Kurtar et al. 2009), winter squash (Kurtar and Balkaya 2010), and CW (Metin et al. 2024).

Conclusion and future perspective

The dihaploidization process remains an accelerated breeding strategy used to rapidly produce 100% pure homozygous lines. It is an essential tool, especially in increasing the efficiency of developing F1 hybrid vegetable cultivars. In addition, the irradiated pollen technique is widely employed in F1 hybrid programs in melon. The irradiation pollen approach can be used in cucumber and squash species to stimulate parthenogenic embryos and produce haploid plants, which can be integrated into breeding programs.

The current study examined the effects of irradiation sources and doses on inducing in situ haploid embryos and in vitro haploid plantlets in potential CW rootstock candidates tolerant to drought stress. The best results were obtained at 300 Gy using the Co^{60} source and 250 Gy using the Cs^{137} source, and haploid plants were raised from all genotypes except the CW8 and CW11 lines. These findings reflected that lines, gamma-ray sources, and irradiation doses influence haploidy frequency in CW. Future haploidization programs should incorporate anther and ovary culture, as well as different media composition, incubation, and culture conditions to improve drought-tolerant CW cultivars since this study only focuses on obtaining haploid plants using irradiated pollen technique on CW rootstock candidates. In addition, future developments in using haploid inducer lines will result in novel approaches to DH studies in the Cucurbitaceae family.

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Author contributions E.S.K. designed the research, writing the original draft, supervision, writing review and editing. D.M., Z.A., and Ü.K. performed the experiments or analyzed the data. M.S., A.R.A. and F.Ç.T. engaged in data curation, formal analysis, and investigation. All authors contributed to the article and approved the submitted version.

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Data availability All datasets generated for this study are included in the article/supplemental tables. The data presented in this study are available on request from the first author.

Declarations

Conflict of interest Ali Ramazan Alan is one of the Associate Editor of the journal at the time of submission. This had no impact on the peer review process and the final decision.

References

Alan AR, Celebi-Toprak F, Lachin A, Yildiz D, Gozen V, Beşirli G (2021) Doubled haploid broccoli (*Brassica Olearacea* var. *Italica*) plants from anther culture. In: Segui-Simarro JM (ed) Doubled Haploid Technology. Methods in Molecular Biology, vol 2288. Humana, New York, NY., pp 201–216. https://doi.org/10.1007/978-1-0716-1335-1_12

Babaoglu D, Türkmen Ö (2017) Some fruit characteristics of genotype of pumpkin (*Cucurbita moschata* Duchesne) with the origin of western Anatolia. J Inst Sci Technol 7(4):11–16

Bagheri L, Lotfi M, Nori M (2021) Production of haploid embryos and plants in Iranian melon (*Cucumis melo* L.) through irradiated pollen-induced parthenogenesis. In Mutation breeding, genetic diversity and crop adaptation to climate change (pp. 127–133). Wallingford UK: CABI. https://doi.org/10.1079/9781789249095.0013

Baktemur G, Yücel NK, Taşkın H, Çömlekçioğlu S, Büyükalaca S (2014) Effects of different genotypes and gamma-ray doses on haploidization using irradiated pollen technique in squash. Turkish J Biology 38:318–327. https://doi.org/10.3906/biy-1309-5

Berber M (2009) Production of haploids in naked seed pumpkins (*Cucurbita pepo* L. var. *styriaca*) by pollination with irradiated pollen. Institute of Natural and Applied Sciences University of Çukurova, MSc Thesis, 144 p

Dal B, Sari N, Solmaz I (2016) Effect of different irradiation sources and doses on haploid embryo induction in Altinbas (*Cucumis melo* L. var. *Inodorus*) melons. Turkish J Agric Forestry 40:552–559. https://doi.org/10.3906/tar-1511-27

Dolcet-Sanjuan R, Claveria E, Garcia-Mas J (2006) Cucumber (*Cucumis sativus* L.) dihaploid line production using in vitro rescue of *in vivo* induced parthenogenic embryos. Acta Hort 725:837–844. https://doi.org/10.17660/ActaHortic.2006.725.116

FAO (2022) Food and Agriculture Organization, crop production statistics. http://www.fao.org/faostat/en/#data/QC. Accessed 17 May 2023

Göçmen M, Aydin E, Simsek I, Sari N, Solmaz I et al (2017) Characterization of some agronomic traits and β-carotene contents of orange fleshed Altinbas melon dihaploid lines. Ekin J Crop Breed Genet 3(1):12–18

Godbole M, Murthy HN (2012) Parthenogenetic haploid plants using gamma irradiated pollen in snap melon (*Cucumis melo* var. *Momordica*). Plant Cell Tissue Organ Cult 109:167–170. https://doi.org/10.1007/s11240-011-0066-9

Goldschmidt H, Breneman JC, Breneman DL (1994) Ionizing radiation therapy indermatology. J Am Acad Dermatol 30:157–182. https://doi.org/10.1016/S0190-9622(94)70014-1

Gonzalo MJ, Claveria E, Monforte AJ, Dolcet-Sanjuan R (2011) Parthenogenic haploids in melon: generation and molecular characterization of a doubled haploid line population. J Am Soc Hortic Sci 136:145–154. https://doi.org/10.21273/JASHS.136.2.145

Guler PG, Sari N, Yetisir H, Yegul M, Kantoglu Y, Kunter B (2017) The effects of genotypes and irradiation doses on haploid embryo induction and plant production in bottle gourd [*Lagenaria siceraria* (Malign) Stanley]. Acta Hort 1151:135–142. https://doi.org/10.17660/ActaHortic.2017.1151.22

Gürsöz N, Abak K, Pitrat M, Rode JC, Dumas de Vaulx R (1991) Obtention of haploid plants induced by irradiated pollen in watermelon (*Citrullus lanatus*). Cucurbit Genet Coop 14:109–110

Košmrlj K, Kastelec D, Bohanec B (2014) Styrian oil pumpkin pollen germinability at higher irradiation doses: optimization of the *in vitro* germination protocol and irradiation procedure. Turkish J Biology 38:516–522. https://doi.org/10.3906/biy-1402-58

Kurtar ES, Balkaya A (2010) Production of in vitro haploid plants from *in situ* induced haploid embryos in winter squash (*Cucurbita maxima* Duchesne ex Lam.) Via irradiated pollen. Plant Cell Tissue Organ Cult 102:267–277. https://doi.org/10.1007/s11240-010-9729-1

Kurtar ES, Seymen M (2021) Gynogenesis in Cucurbita species. In: Segui-Simarro JM (ed) Doubled Haploid Technology. Methods in Molecular Biology, vol 2289. Humana, New York, pp 123–133. https://doi.org/10.1007/978-1-0716-1331-3_8

Kurtar ES, Sarı N, Abak K (2002) Obtention of haploid embryos and plants through irradiated pollen technique in squash (*Cucurbita pepo* L). Euphytica 127:335–344. https://doi.org/10.1023/A:1020343900419

Kurtar ES, Balkaya A, Ozbakir M, Ofluoglu T (2009) Induction of haploid embryo and plant regeneration via irradiated pollen technique in pumpkin (*Cucurbita moschata* Duchesne ex. Poir). Afr J Biotechnol 8:5944–5951

Kurtar ES, Seymen M, Kal Ü (2020) An overview of doubled haploid plant production in Cucurbita species. Yuzuncu Yıl Univ J Agricultural Sci 29(2):510–520. https://doi.org/10.29133/yyutbd.741087

Kurtar ES, Seymen M, Çetin AN, Türkmen Ö (2021) Dihaploidization in promising summer squash genotypes (*Cucurbita pepo* L.) via irradiated pollen technique. Yuzuncu Yıl UniversityJournal Agricultural Sci 31(1):42–51. https://doi.org/10.29133/yyutbd.800475

Kurtar ES, Seymen M, Yavuz D, Acar B, Metin D et al (2024) Morphophysiological and biochemical investigation of the potential of citron watermelon (*Citrulluslanatus* var. *citroides*) rootstock under different irrigation regimes. Horticulture Environment and Biotechnology 1–15. https://doi.org/10.1007/s13580-024-00627-1

Lim W, Earle ED (2008) Effect of in vitro and in vivo colchicine treatments on pollen production and fruit set of melon plants obtained by pollination with irradiated pollen. Plant Cell Tissue Organ Cult 95:115–124. https://doi.org/10.1007/s11240-008-9422-9

Liu D, Xie L, Xiao W, Xie T, Wu F et al (2024) Production of haploid plants in wax gourd through parthenogenesis induced by gamma-irradiated pollen. Sci Hort 336:113450. https://doi.org/10.1016/j.scienta.2024.113450

Lotfi M, Kashi A, Onsinejad R (1999) Induction of parthenogenetic embryos by irradiated pollen in cucumber. Acta Hort 492:323–328. https://doi.org/10.17660/ActaHortic.1999.492.44

Lotfi M, Alan AR, Henning MJ, Jahn MM, Earle ED (2003) Production of haploid and doubled haploid plants of melon (*Cucumis melo* L.) for use in breeding for multiple virus resistance. Plant Cell Rep 21:1121–1128. https://doi.org/10.1007/s00299-003-0636-3

Malambane G, Batlang U, Ramolekwa K, Tsujimoto H, Akashi K (2021) Growth chamber and field evaluation of physiological factors of two watermelon genotypes. Plant Stress 2:100017. https://doi.org/10.1016/j.stress

Mandizvo T, Odindo AO, Mashilo J, Magwaza LS (2022) Drought tolerance assessment of citron watermelon (*Citrullus lanatus* var.

citroides (L.H. Bailey) Mansf. Ex Greb.) Accessions based on morphological and physiological traits. Plant Physiol Biochem 80:106–123. https://doi.org/10.1016/j.plaphy.2022.03.037

Metin D, Atakul Z, Kurtar ES, Seymen M, Alan AR et al (2024) Callogenesis, embryogenesis, and plantlet initiation in citron watermelon (*Citrullus lanatus* var. *citroides*) via anther and unfertilized ovary culture. Sci Hort 113493. https://doi.org/10.1016/j.scienta.2024.113493

Murashige T, Skoog FA (1962) Revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497

Parkash C, Bhatia R, Munshi AD, Rao M, Subhasmita S et al (2024) An efficient and easy-to-use protocol for induction of haploids in cucumber through parthenogenic embryo development. Bot Stud 65(1):1–17. https://doi.org/10.1186/s40529-024-00436-w

Reshmika PK, Pradeepkumar T, Paikattumana S, Iyer KS, Koorathodi V et al (2024) Use of irradiated pollen technique to recover haploids in bitter gourd. Indian J Hortic 81(01):37–42

Salehian H, Shahnazi S, Nazari M (2023) Production of doubled haploid plants in cucumber (Cucumis sativus L.) via parthenogenesis. Vitro Cell Dev Biology-Plant 59(4):467–474. https://doi.org/10.1007/s11627-023-10368-y

Sari N (1994) Effect of genotype and season on the obtention of haploid plants by irradiated pollen in watermelon and alternatives to the irradiation. PhD Thesis, University of Çukurova

Sari N, Solmaz I (2021) Doubled haploid production in watermelon. In: Segui-Simarro J.M (eds) Doubled Haploid Technology. Methods in Molecular Biology, vol 2289. Humana, New York, NY. 97–110. https://doi.org/10.1007/978-1-0716-1331-3_6

Sari N, Abak K, Pitrat M, Rode JC, Dumas de Vaulx R (1994) Induction of parthenogenetic haploid embryos after pollination by irradiated pollen in watermelon. HortScience 29:1189–1190

Sari N, Abak K, Pitrat M (1999) Comparison of ploidy level screening methods in watermelon (*Citrullus lanatus* (Thunb.) Matsum. And Nakai). Sci Hort 82:265–277. https://doi.org/10.1016/S0304-4238(99)00077-1

Sari N, Solmaz I, Yetisir H, Ekiz H, Yucel S (2009) New fusarium wilt resistant melon (*Cucumis melo* var. *cantalupensis*) varieties developed by dihaploidization: Sari F1, Yetisir F1, Solmaz F1, Emin F1 and Yucel F1. In IV International Symposium on Cucurbits 871 (pp. 267–272). https://doi.org/10.17660/ActaHortic.2010.871.35

Seymen M, Yavuz D, Ercan M, Akbulut M, Çoklar H (2021) Effect of wild watermelon rootstocks and water stress on chemial properties of watermelon fruits. Hortic Environ Botechnol 62:411–422. https://doi.org/10.1007/s13580-020-00329-4

Şimşek D, Doğan S, Öz D, Ellialtıoğlu ŞŞ (2023) Obtaining pure lines in melon using the irradiated pollen technique. Areo Seed, HEKTAŞ, Seed Technologies Center – Antalya, Tomato Breeding Programs, Project No, p 7

Sun Y, Mei S, Peng J, Zhang L, Nie Q et al (2006) Induced haploid plants after pollination by irradiated pollen in *Cucumis melo* L. Hubei Agricultural Sci 4:98–100

Taner KY, Yanmaz R, Kunter B (2000) The effects of irradiation dose and harvest period on haploid plant formation via irradiated pollen in snake cucumber (*Cucumis melo* var. *flexuosus* Naud.). III[rd] National Vegetable Culture Symp, Isparta-Turkey, pp. 177–181. 340

Taşkın H, Yücel NK, Baktemur G, Çömlekçioğlu S, Büyükalaca S (2013) Effects of different genotypes and gamma ray doses on haploidization with irradiated pollen technique in watermelon (*Citrullus lanatus* L). Can J Plant Sci 93:1165–1168. https://doi.org/10.4141/cjps2013-059

Teppner H (2004) Notes on Lagenaria and Cucurbita (Cucurbitaceae)-review and new contribution. Phyton (Horn Austria) 44(2):245–308

Thies JA, Buckner S, Horry M, Hassell R, Levi A (2015) Influence of *Citrullus lanatus* var. *citroides* rootstocks and their F_1 hybrids on yield and response to root-knot nematode, *Meloidogyne incognita*, in grafted watermelon. HortScience 50 (1): 9–12. https://doi.org/10.21273/HORTSCI.50.1.9

Wechter WP, Kousik C, McMillan M, Levi A (2012) Identification of resistance to Fusarium oxysporum f. sp. niveum race 2 in Citrullus lanatus var. citroides plant introductions. HortScience 47(3):334–338. https://doi.org/10.21273/HORTSCI.47.3.334

Yapıcı B, Gürsoy E, Yıldız BN, İpek E, Kavak S et al (2023) Effect of cesium- sourced gamma irradiation on haploidization in powdery mildew and zymv resistant summer summer summer (*Cucurbita pepo* L.) lines. International Ege Agriculture Congress, November 01–03, 2023/İzmir-Antalya, Türkiye, 560–580, ISBN: 978-625-367-426-7

Yavuz D, Baştaş KK, Seymen M, Yavuz N, Kurtar ES et al (2023) Role of ACC deaminase-producing rhizobacteria in alleviation of water stress in watermelon. Sci Hort 321:112288. https://doi.org/10.1016/j.scienta.2023.112288

Zhao Q, Zhang M, Qi H, Yang X (2023) Haploid induction and plant production in bottle gourd by pollination with gamma-irradiated pollen. Plant Cell Tissue Ogan Cult. 152:331–338. https://doi.org/10.1007/s11240-022-02410-5