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Applications of Chromosome Doubling in Haploid Squashes and Pumpkins (*Cucurbita* spp.)(§)

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ABSTRACT

Squashes and pumpkins are important vegetable species belonging to the genus *Cucurbita* of the family Cucurbitaceae. The genus Cucurbita (2n=40) comprises five cultivated species, the most economically important of which are *Cucurbita pepo* L., *C. maxima* Duch., and *C. moschata* Duch. The doubled haploidization technique, which allows the rapid and efficient obtaining of pure lines in variety breeding programs in many plant species, is also successfully applied in the *Cucurbita* genus. The irradiated pollen technique yields successful results in all three squash species in obtaining haploid plants. Successful applications have been made in projects conducted at our company to obtain haploid plants in squash. However, it is not possible to propose a practical protocol for chromosome doubling in squash and obtaining doubled haploid (DH) plants from haploid plants. Although the literature contains successful applications and reports of pure-line seeds, the doubling step remains a key challenge in the production of pure lines in squash. For this purpose, studies were conducted on chromosome doubling in haploid squash plants under *in vitro* and *in vivo* conditions. Concentration and duration of colchicine applications were tested, and stomatal examinations revealed diploid plants. The most effective method was determined to be a 3-hour application period using a 0.5% colchicine dose used in acclimated young haploid seedlings at the 3-4 leaf stage. Plants grown from this treatment were self-pollinated to obtain pure-line seeds. However, optimization studies on chromosome doubling in squash varieties are still needed.

Keywords**:** Squashes and pumpkins, haploid, *Cucurbita pepo, C. moschata, C. maxima*, colchicine, chromosome doubling

Introduction

Squashes and pumpkins, belonging to the genus *Cucurbita* of the Cucurbitaceae family, are among the most important vegetable crops. Native to the Americas, their domestication coincides with the beginnings of settled agriculture. Among the five cultivated species, *Cucurbita pepo* L., *C. maxima* Duch., and *C. moschata* Duch. are the most economically significant (Whitaker and Davis, 1962; Robinson and Decker-Walters, 1997). *C. pepo* and *C. maxima* are generally adapted to temperate climates, whereas *C. moschata* thrives better in tropical and subtropical regions. Globally, squash is cultivated on approximately 2 million hectares, producing around 27 million tons annually. China, India, and Russia are the leading producers, while Türkiye ranks eighth with an average annual production between 700.000 and 800.000 tons (Anonymous, 2025a). Approximately 14% of Türkiye's total squash production is allocated to foreign trade, reflecting the crop's considerable contribution to the country's agricultural exports (Anonymous, 2025b).

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The domestication of *Cucurbita pepo*, native to Mexico and Central America, dates back approximately 10,000 years (Smith, 1997), and it is regarded as one of the earliest cultivated crops of the New World (Nee, 1990). Historical evidence suggests that *C. moschata* was domesticated around 3000 BC in Peru and 2000 BC in Guatemala (Smith, 2005), while *C. maxima* originated along the Peruvian coast around 2000 BC (Ferriol and Picó, 2008). Cultivated *Cucurbita* species subsequently spread to the Old World and became widely grown after the 16th century (Paris, 1989). Although no wild *Cucurbita* species are found in Türkiye, the genus exhibits substantial genetic diversity within the region (Balkaya and Karaağaç, 2006). According to Harlan (1951), Anatolia serves as a micro-center of diversity for several Cucurbitaceae species, including *C. melo* L., *C. sativus* L., *C. moschata* Duch., and *C. pepo* L.

Due to their monoecious floral structure, cucurbits exhibit a high degree of cross-pollination, with rates typically ranging from 60%-80%, depending on pollinator activity and the genetic background of the population (Abbey, 2016). This high outcrossing rate considerably prolongs the time required to achieve homozygosity during the development of parents inbred lines for F_1 hybrid breeding. Even after several generations of selfing, residual genetic segregation may persist. While self-pollinated species usually reach acceptable homozygosity within 4-6 generations, this process may extend to 8-10 generations in cucurbits (Kurtar et al., 2024). Kesici et al. (2000) carried out 11 successive generations of selfing to develop parental lines for hybrid breeding in summer squash. While inbreeding depression was not evident, the authors noted a slight reduction in plant habit and fruit size, which can complicate the characterization and stabilization of desirable line traits.

Within plant tissue culture, the doubled haploid (DH) approach, also known as gametic embryogenesis, has become increasingly important in modern plant breeding. It is particularly valuable for rapidly developing high-performing hybrid varieties in both field crops and vegetable seed production. The doubled haploidization process significantly shortens the time required for line purification-a critical step in F_1 hybrid development that would otherwise necessitate 6-8 generations of selfing in highly outcrossing species-reducing it to just 1-2 years. This method not only conserves time, labor, and resources but also accelerates the introduction of new varieties to the market. Moreover, DH technology enables the production of fully homozygous "pure lines," which cannot be achieved through conventional repeated selfing (Kurtar et al., 2020).

Several factors influence, and at times constrain, the successful application of the DH technique in plant breeding. Beyond the limitations encountered during haploid plant production, a major challenge lies in restoring fertility via chromosome doubling. Achieving doubled haploid plants with a diploid chromosome complement represents a crucial and indispensable step in the process. Chromosome doubling is carried out in haploid (n) plants, as determined through stomatal observations and flow cytometry analyses, either under *in vitro* or *in vivo* conditions (Kurtar et al., 2024).

Colchicine is the most commonly employed antimitotic agent for chromosome doubling in cucurbits (Lim and Earle, 2009; Galazka and Niemirowicz-Szczytt, 2013; Aslam et al., 2017). Recent comprehensive reviews have highlighted that chromosome doubling remains one of the major bottlenecks in doubled haploid (DH) technology and polyploid breeding, particularly in recalcitrant vegetable species. Fomicheva et al. (2024) emphasized that the success of chromosome doubling is strongly influenced by species, genotype, developmental stage, colchicine concentration, exposure time, and application method, making it difficult to establish a universal protocol. Focusing specifically on the Cucurbitaceae family, Ermolaev et al. (2026) reported that colchicine remains the principal antimitotic agent for chromosome doubling in *Cucurbita*, *Cucumis*, and *Citrullus* species, although alternative compounds such as oryzalin and other dinitroaniline herbicides have also been investigated. Nevertheless, chromosome doubling efficiency is still highly variable, and the frequent occurrence of mixoploid and chimeric plants indicates that reliable, species-specific protocols are still lacking, particularly in *Cucurbita*. Likewise, Singh et al. (2025) concluded that although colchicine continues to be the standard chemical for induced polyploidization because of its effectiveness in disrupting spindle formation during mitosis, alternative antimitotic agents, including oryzalin, trifluralin, and amiprophos-methyl, may improve chromosome doubling efficiency or reduce phytotoxic effects in certain species. Collectively, these recent studies demonstrate that further optimization of chromosome doubling protocols remains essential for efficient DH production in cucurbit breeding programs.

Various chromosome duplication strategies have been developed, including immersion of entire plantlets or nodal explants (Sarı and Abak, 1996; Çağlar and Abak, 1997), treatments applied directly to apical meristems (Nikolova and Niemirowicz-Szczytt, 1996), shoot tips (Yetişir and Sarı, 2003; Solmaz et al., 2011), as well as lateral shoots. These approaches have

been widely adopted in cucurbit breeding programs to induce doubled haploidy efficiently.

Despite considerable progress in haploid production techniques, chromosome doubling remains one of the least optimized steps in doubled haploid (DH) technology for *Cucurbita* species. Most previous studies have focused on haploid induction, whereas practical and reproducible protocols for obtaining fertile DH plants through chromosome doubling are still limited. Therefore, the development of efficient chromosome doubling strategies is essential for accelerating DH-based breeding in cucurbit crops. In the present study, haploid squash plants derived from parthenogenetic embryos induced by irradiated pollen of *C. pepo*, *C. maxima*, and *C. moschata* were subjected to both *in vitro* and *in vivo* colchicine treatments at different developmental stages. The novelty of this study lies in the comparative evaluation of chromosome doubling approaches under both *in vitro* and *in vivo* conditions across economically important *Cucurbita* species, with the aim of identifying a practical protocol for obtaining fertile doubled haploid plants.

Materials and Methods

The experiments were carried out at the Petektar Seed R&D Center, which comprises a research greenhouse and plant biotechnology laboratories located in the Kurşunlu district of Antalya. Plant materials consisted of breeding lines and experimental genotypes of *Cucurbita pepo*, *C. maxima*, and *C. moschata* maintained in the breeding program of Petektar Seed Co. For the *in vitro* doubled haploid (DH) experiments, breeding lines from ongoing breeding projects were used. These materials consisted of segregating F_3 populations selected based on desirable fruit characteristics and cold tolerance. Haploid plants were obtained from parthenogenetic embryos induced by irradiated pollen.

Obtaining haploid plants in Cucurbita spp.

Male flowers were collected one day before anthesis and irradiated with 150 Gy using a Cobalt-60 gamma source (Baktemur et al., 2014). Pollen, although generatively inactive, retained its germination capacity and was used to pollinate female flowers isolated one day earlier. Fruits were harvested at 3-4 weeks of age, before reaching full maturity, following successful pollination. The harvested fruits were dissected under sterile conditions, and individual seeds were carefully examined. Embryos excised aseptically were cultured on nutrient media and successfully regenerated into plants (Kurtar and Seymen, 2021; Yiğit et al., 2023). Further details and results concerning this stage have been previously described by Yapıcı et al. (2023).

In vitro colchicine treatments and ploidy estimation

Parthenogenetic embryos were directly cultured on nutrient media supplemented with colchicine (Figure 1). Three colchicine concentrations (0.3%, 0.5%, and 0.6%) were incorporated into MS (Murashige and Skoog, 1962) media (MS salts and vitamins + 0.01 mg/L IAA + 0.01 mg/L GA_3 + 3% sucrose + 0.7% agar, pH 5.8). Following autoclaving and placement in the culture cabinet, colchicine was aseptically incorporated into the nutrient media via filter sterilization. For each treatment, 200 haploid embryos of *C. pepo* were cultured. After germination and one month of growth (Figure 1), the embryos were acclimatized and successfully developed into healthy seedlings in the incubation room for 21 days (Figure 2).

Chloroplast numbers in stomatal guard cells were determined from leaf sections. Fresh leaves were initially cleared in Carnoy's solution (3 parts ethanol: 1 part glacial acetic acid), rinsed in sterile water for 2-5 minutes, and subsequently stained with 1% I-KI solution for 30 seconds. For each sample, chloroplast counts were performed on 30 stomata, and observations were made under a microscope at 400× magnification (Doğan et al., 2023).

In vivo colchicine treatments

Haploid *C. maxima* and *C. moschata* plantlets obtained through irradiated pollen-induced parthenogenetic embryogenesis and subsequently acclimatized were treated with colchicine in the incubation room. *In vivo* application of antimitotic agents was carried out following modified protocols of Nikolova and Niemirowicz-Szczytt (1996) and Kurtar (2018). Small cotton pieces, soaked in the doubling solutions, were placed on the apical regions of seedlings at the 4-5 true-leaf stage. The cotton was then covered with aluminum foil and stretch film to prevent light exposure and evaporation (Figure 3). For this purpose, a 0.5% colchicine solution was applied for 3 hours, after which the shoot tips were rinsed with sterile water. The treatment was repeated a second time following a one-day interval. Treated seedlings were maintained under a daily photoperiod at 25 ± 2°C during the day and 18 ± 2°C at night. After 15 days, the plants were transferred to the greenhouse and established in their designated positions, where they were grown under controlled conditions.

Selfing, fruit set, and seed set

Fruit and seed set observations were used to assess the reproductive potential of the doubled haploids (DHs). One day before anthesis, both female and male flowers were isolated using cloth bags. The following morning, female flowers were self-pollinated and re-

isolated to prevent unintended pollen contamination. Cloth bags were removed on the second or third day after pollination. For each DH plant, at least one female flower was pollinated. However, self-pollination was not always possible, as in some cases, both male and female flowers were not present on the same plant at the same time. Fruit set was recorded as the number of plants bearing fruit, while seed set was evaluated based on the average number of fully developed seeds per fruit.

Results and Discussion

In vitro colchicine treatments and ploidy estimation

In treatments with 0.3% colchicine, 8% of the embryos failed to develop into complete plants and could not be transferred to soil. Similarly, 12% and 14% of the embryos in 0.5% and 0.6% colchicine treatments, respectively, did not survive to full plantlets. All other plants were successfully acclimatized and grown in the growth chamber until they reached the 4-5 leaf stage.

As a result of the treatments applied to obtain doubled haploid plants directly from haploid individuals through colchicine supplementation in the nutrient media, mixoploid *C. pepo* plants were obtained in all treatments without statistically significant differences. The stomata and chloroplast numbers in the leaves of these plants were examined following the protocol of Doğan et al. (2023). Even within three different regions of a single leaf, variations in chloroplast numbers were observed. When sampling was increased across leaves on the same plant, similar variation was consistently observed throughout the entire plant.

Antimitotic agents are commonly recognized as metaphase inhibitors, exerting their effects primarily during the metaphase stage of cell division. Compounds such as colchicine, colcemid, vinblastine, acenaphthene, dinitroanilines, phosphoroamidates, pyridines, benzamides, and benzoic acid derivatives interfere with metaphase progression and induce chromosome doubling. In particular, colchicine disrupts microtubule dynamics, thereby preventing cell division and leading to chromosome duplication (Dewitte and Murray, 2003; Vaughn, 2006; Dhooghe et al., 2011). Exposure of haploid plants to colchicine, oryzalin, trifluralin, and other antimitotic chemicals *in vitro* or *in vivo* results in chromosome doubling (Kurtar, 2018; Vural et al., 2019). Colchicine acts by inhibiting microtubule formation, thus altering the cell division process in plant cells. Recent reviews have confirmed that colchicine remains the most widely used antimitotic agent in doubled haploid technology because of its effectiveness in disrupting spindle formation during mitosis. However, chromosome doubling efficiency is strongly influenced by species, genotype, developmental stage, treatment duration, and application method. Alternative antimitotic agents, including oryzalin, trifluralin, and amiprophos-methyl, have also been investigated to improve chromosome doubling efficiency or reduce phytotoxic effects, although no universally applicable protocol has yet been established for vegetable crops, particularly for recalcitrant members of the Cucurbitaceae (Fomicheva et al., 2024; Ermolaev et al., 2026; Singh et al., 2025). Marangoz et al. (2025) applied colchicine at concentrations of 0.3%, 0.4%, and 0.6% in both semi-solid and double-layer media for 14 and 21 days in pepper anther cultures. They reported that colchicine significantly enhances the production of spontaneous doubled haploid plants, with the most effective treatment being 0.6% colchicine for 21 days in semi-solid medium.

The size of stomatal guard cells and the number of chloroplasts within these cells show a strong correlation with the ploidy level of plants, indicating whether they are haploid, diploid, or polyploid (Ellialtıoğlu et al., 2002; Tepe et al., 2002; Bat et al., 2021). Therefore, chloroplast numbers in the stomatal guard cells were examined in plantlets obtained following *in vitro* colchicine treatment of summer squash (*C. pepo*). Based on reference values established for *C. pepo*, the average number of chloroplasts per stoma was 5.2 ± 1.3 in haploid plants and 9.8 ± 0.7 in diploid plants. In acclimatized plantlets, chloroplast counts were recorded from five different leaf regions. The mean chloroplast number per stoma increased from 6.6 ± 1.2 in the 0.3% colchicine treatment to 8.3 ± 0.9 and 10.7 ± 1.6 in the 0.5% and 0.6% treatments, respectively. This dose-dependent increase indicates the presence of cells with duplicated chromosomes and, particularly at the highest colchicine concentration, cells with even higher ploidy levels. However, the coexistence of cells exhibiting both haploid and diploid characteristics clearly indicates the presence of mixoploidy. Cytological observations confirmed that none of the regenerated plants were entirely haploid or fully diploid; instead, all regenerated plantlets exhibited varying degrees of mixoploidy. As illustrated in Figures 4 and 5, microscopic observations revealed chloroplast distributions characteristic of haploid, diploid, and mixoploid tissues within the same regenerated plants.

Nevertheless, the occurrence of mixoploid plants following colchicine treatment is not unique to *Cucurbita* and has been reported in several crop species. Chinnathambi et al. (2023) obtained regenerated marigold plants with mixed ploidy following colchicine

treatment of androgenic haploids, while Klíma et al. (2024) reported a high proportion of mixoploid regenerants after colchicine application in microspore-derived swede plants. Similarly, Fomicheva et al. (2025) observed the formation of mixoploid regenerants during chromosome doubling of carrot haploid embryos using colchicine and trifluralin. Collectively, these studies demonstrate that chromosome doubling induced by antimitotic agents is rarely uniform throughout the entire plant because different meristematic regions or cell layers may respond differently to treatment. Consequently, the formation of mixoploid plants should be regarded as a common biological consequence of chromosome doubling rather than a species-specific phenomenon. The results obtained in the present study are therefore consistent with recent findings across several plant families and with the conclusions of Fomicheva et al. (2024) and Ermolaev et al. (2026), who identified incomplete chromosome doubling and the frequent occurrence of mixoploid plants as major bottlenecks in doubled haploid production, particularly in the Cucurbitaceae. Further optimization of chromosome doubling protocols will therefore be essential to increase the frequency of doubled, fertile plants in *Cucurbita*.

When the mixoploid squash plants were grown in the greenhouse and induced to flower, frequent occurrences of male flowers lacking pollen and morphologically abnormal flowers with malformed stigmas were observed (Figure 6). In cases where pollen-producing male flowers were used for self-pollination or for pollinating female flowers of control diploid plants, no fruit set was achieved. Moreover, in many plants, male and female flowers did not develop simultaneously, which further limited successful fertilization. Indeed, Yuan et al. (2015) reported in cabbage and broccoli, and Fomicheva et al. (2025) in carrot, that colchicine treatments applied to haploid plants obtained via androgenesis can result in the development of mixoploid individuals, and that obtaining seeds from these plants is often a limiting factor. Therefore, under the experimental conditions of this study, *in vitro* colchicine treatments were not sufficient to achieve successful DH seed formation in squash.

***In vivo* colchicine treatments**

In vivo chromosome doubling of winter squash and pumpkin haploid lines was performed through colchicine treatments applied to the apical parts of acclimatized haploid plantlets. Following the protocol of Kurtar (2018) and considering all experimental results obtained throughout the study, the colchicine treatment was optimized as 0.5% colchicine applied for 3 hours and repeated twice at one-day intervals. This treatment successfully produced doubled haploid (DH) plants. Three fertile DH plants with viable seeds were obtained from each self-pollinated *C. maxima* and *C. moschata* genotype. Out of 20 acclimatized haploid plants treated per species, a 15% chromosome doubling rate was achieved (Figure 7), which is comparable to the values previously reported by Kurtar (2018).

The successful recovery of fertile DH plants in the present study confirms that chromosome doubling efficiency depends not only on the antimitotic agent itself but also on genotype, developmental stage, treatment duration, and application strategy. Recent reviews have emphasized that these factors interact to determine chromosome doubling success, making protocol optimization more critical than colchicine concentration alone (Fomicheva et al., 2024). The use of acclimatized haploid seedlings at the 4-5 leaf stage in the present study appears to have contributed to the successful recovery of fertile DH plants, highlighting the importance of selecting an appropriate developmental stage for chromosome doubling.

Cucurbita species have long been regarded as recalcitrant crops with respect to both plant regeneration and chromosome doubling (Kathiravan et al., 2006; Ananthakrishnan et al., 2007; Ebrahimzadeh et al., 2018). These challenges are reflected in the generally low responsiveness of cucurbits to spontaneous chromosome doubling and *in vitro* polyploid induction (Hooghvorst and Nogués, 2020). Consequently, obtaining fertile doubled haploid plants remains considerably more difficult in *Cucurbita* than in many other vegetable crops.

Previous studies have successfully applied colchicine for chromosome doubling in several cucurbit and solanaceous species, including *Cucumis melo* (Sauton and Dumas de Vaulx, 1987; Lim and Earle, 2009), *Citrullus lanatus* (Sarı and Abak, 1996), *Cucumis sativus* (Çağlar and Abak, 1997), pepper (Vural et al., 2019; Marangoz et al., 2025), and other vegetable crops. However, because of the particular recalcitrance of *Cucurbita*, alternative antimitotic agents such as oryzalin and trifluralin have also been evaluated (Kurtar, 2018; Ebrahimzadeh et al., 2018). Recent reviews indicate that although these compounds may reduce phytotoxicity or improve chromosome doubling efficiency in certain species, colchicine remains the principal antimitotic agent in cucurbit breeding because of its broad applicability and consistent performance (Ermolaev et al., 2026; Singh et al., 2025). Therefore, future improvements in chromosome doubling efficiency are more likely to be achieved through optimization of treatment protocols than by replacing colchicine itself.

Colchicine has been applied at concentrations ranging from 0.05 to 5 g L^{-1}, depending on species and application protocol (Fomicheva et al., 2024). The present study demonstrated that a 0.5% colchicine treatment for 3 hours, repeated twice, was sufficient to recover fertile DH plants in both *C. maxima* and *C. moschata*. Although additional optimization involving different concentrations, exposure periods, or repeated applications may further improve chromosome doubling efficiency (Kurtar, 2018), the present protocol provides a practical and reproducible approach for obtaining fertile DH plants in economically important *Cucurbita* species.

The number of seeds harvested per DH *Cucurbita* plant ranged from 32 to 166. Despite the well-known recalcitrance of cucurbits to chromosome doubling, fertile DH plants were successfully obtained. The resulting DH seed populations are currently being evaluated under protected cultivation for genetic uniformity and phenotypic stability. These results demonstrate that optimized *in vivo* colchicine treatment can effectively overcome one of the major bottlenecks in doubled haploid technology and provide a reliable strategy for accelerating breeding programs in *Cucurbita*.

Conclusions

This study demonstrated that chromosome doubling efficiency in *Cucurbita* is strongly influenced by the method and developmental stage at which colchicine is applied. While *in vitro* colchicine treatments resulted predominantly in mixoploid plants that failed to produce fertile seeds, *in vivo* application to acclimatized haploid plantlets successfully generated fertile doubled haploid plants in both *C. maxima* and *C. moschata*. The optimized protocol, consisting of two applications of 0.5% colchicine for 3 hours at one-day intervals, proved to be an effective and reproducible approach for chromosome doubling under the conditions of this study. These findings confirm that optimization of treatment strategy, rather than increasing colchicine concentration alone, is critical for improving chromosome doubling efficiency in recalcitrant *Cucurbita* species. The developed protocol provides a practical tool for accelerating doubled haploid production and may contribute to the development of pure lines and the shortening of breeding cycles in cucurbit improvement programs. Future studies should evaluate the applicability of this protocol to a broader range of genotypes and investigate alternative antimitotic agents and genotype-specific optimization strategies to further enhance chromosome doubling efficiency.

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The authors used OpenAI ChatGPT solely to improve the English language, grammar, and readability of the manuscript. The AI tool was not used to generate scientific content, analyze data, interpret results, or draw conclusions. All scientific content and conclusions are entirely the responsibility of the authors.

Ethical Statement

A preliminary version of this study was presented as a poster at the "V International Plant Breeding Congress, 1-5 December 2025, Sherwood Exclusive Lara, Antalya, Türkiye". The present manuscript is an expanded and revised version prepared for full publication.

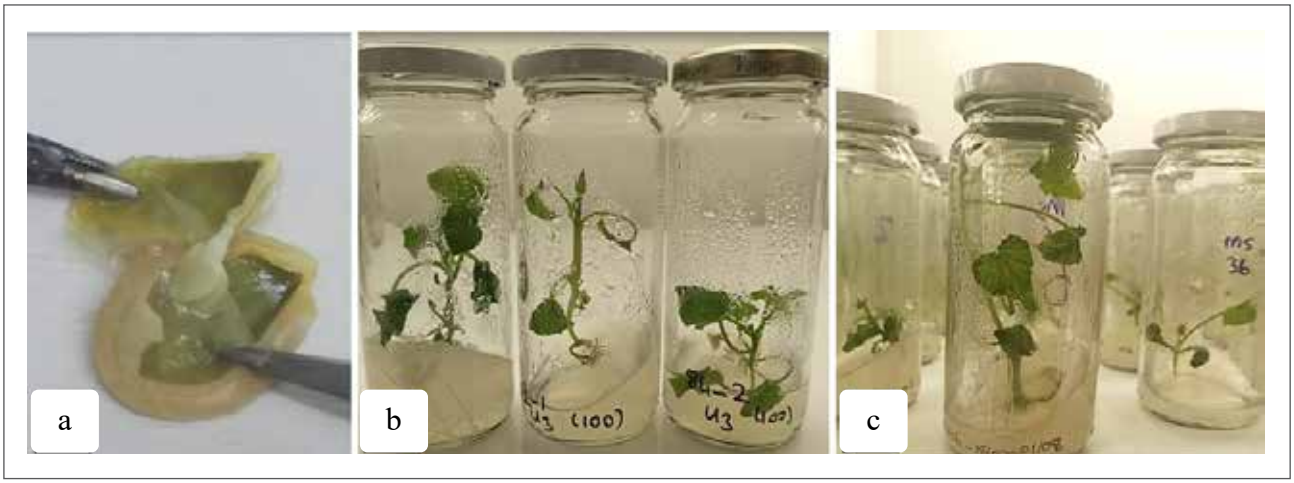

Figure 1. *In vitro* colchicine treatments. (a) Isolation and culture of parthenogenetic embryos, (b) Haploid squash plantlets grown for 21 days on media containing different colchicine concentrations, (c) Plantlets transferred to MS medium and further developed.

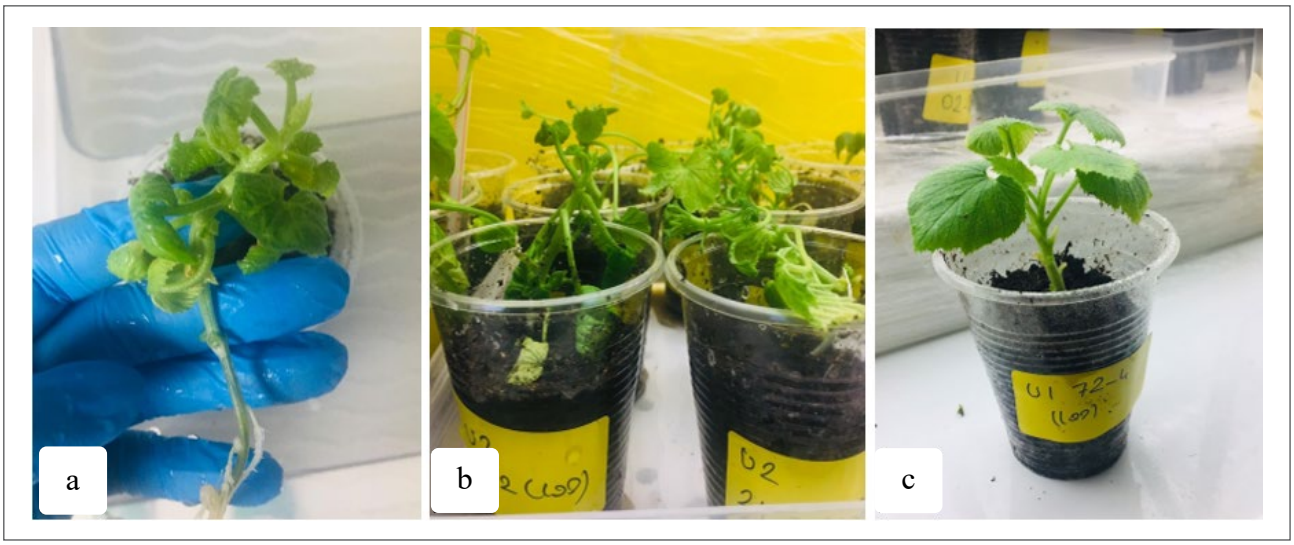

Figure 2. Acclimatization of squash plantlets obtained after colchicine treatment. (a) A plantlet was removed from the glass jar and cleaned of agar, (b) Plantlets transplanted into sterile soil and placed in a mini greenhouse for acclimatization, (c) A fully acclimatized squash plantlet showing healthy growth.

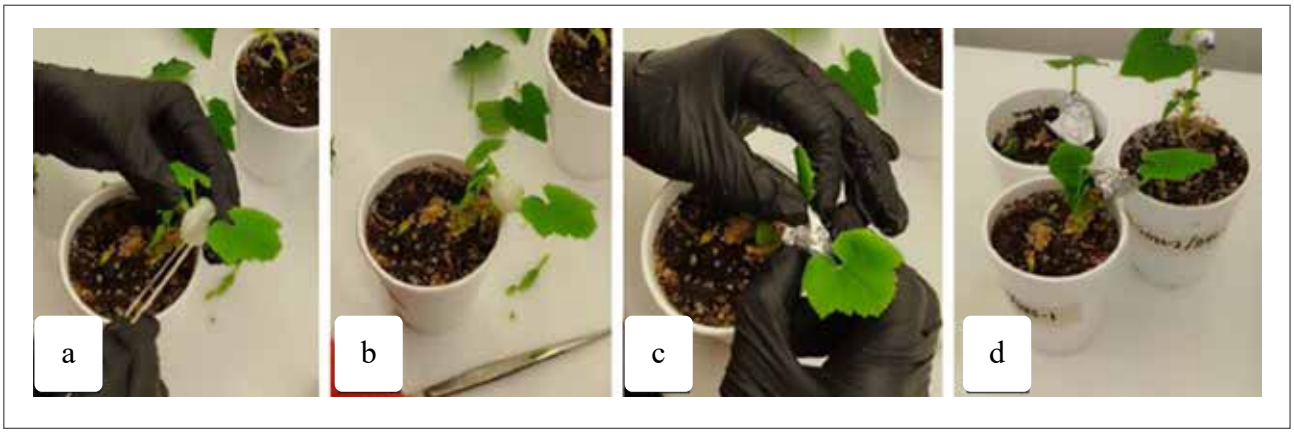

Figure 3. *In vivo* colchicine treatments. (a, b) Placement of cotton imbibed with colchicine solution on the shoot apex, (c, d) Covering the cotton with aluminum foil and leaving it for 3 hours.

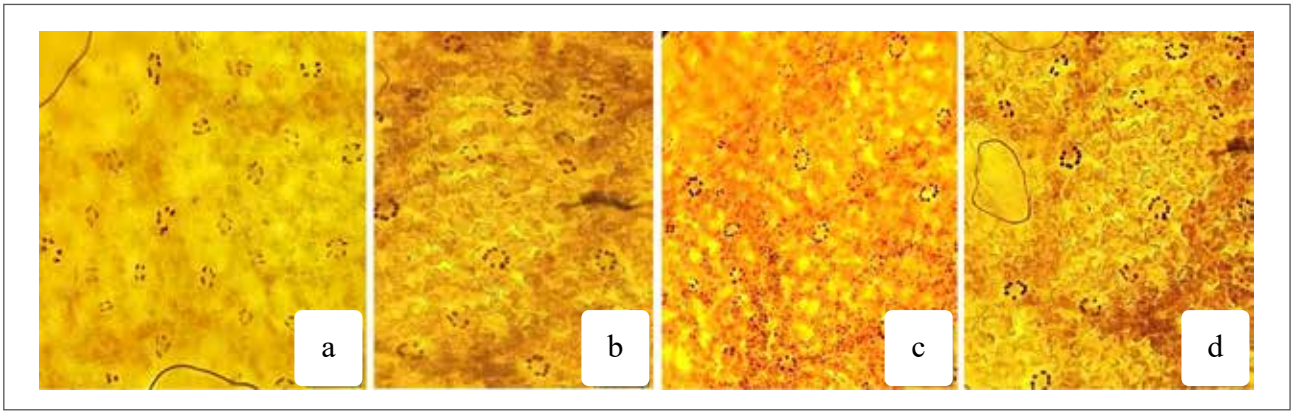

Figure 4. Chloroplast patterns associated with ploidy levels in different plants. (a) Chloroplasts in stomatal cells of a haploid squash leaf, (b-d) Chloroplasts in stomatal cells of a diploid squash leaf.

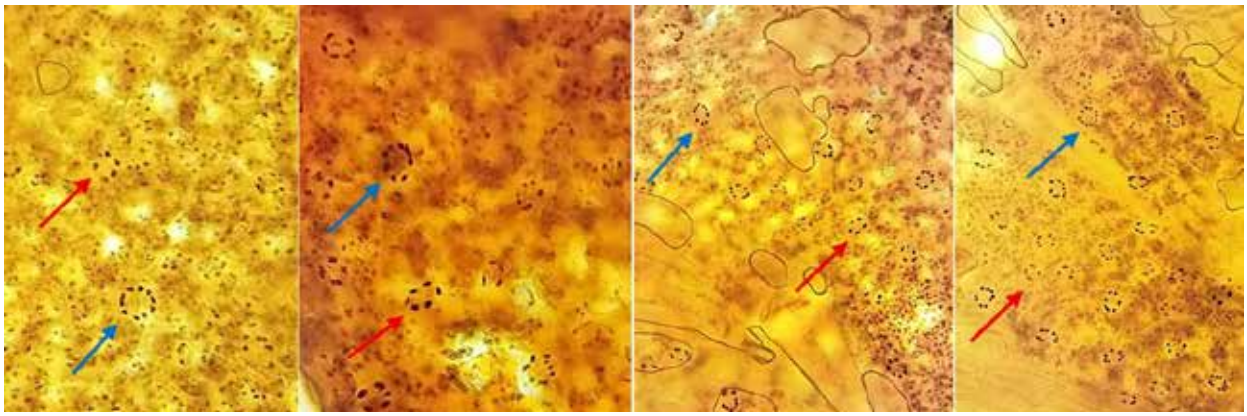

Figure 5. Chloroplast distribution in stomatal cells of mixoploid squash plants (blue arrows: chloroplasts in diploid cells; red arrows: chloroplasts in haploid cells).

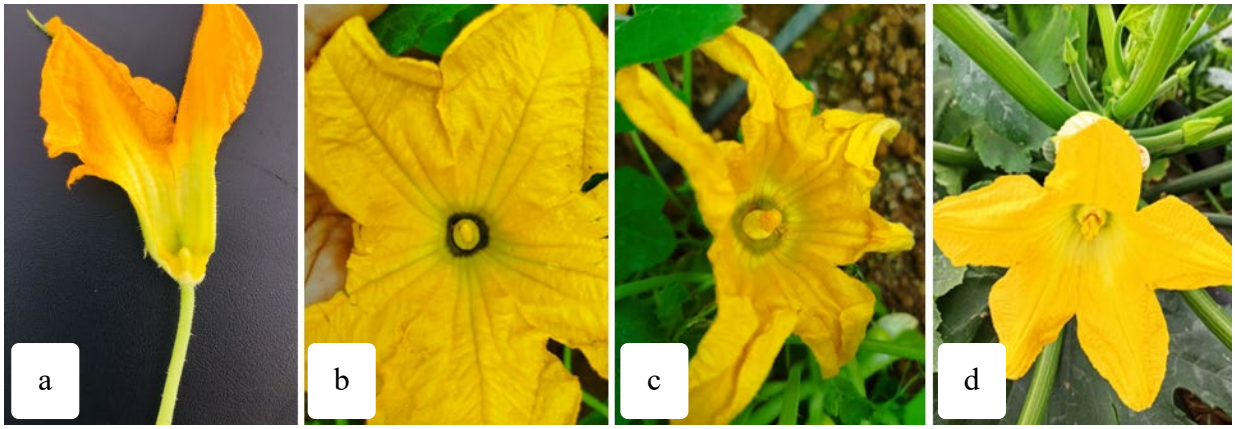

Figure 6. Abnormal flower structures observed in mixoploid squash plants (a, b) Male flowers without pollen, (c) Male flower with pollen but morphologically abnormal and incapable of fertilization, (d) Female flower exhibiting stigma anomalies.

Figure 7. Fruits obtained from self-pollination of healthy DH plants derived from *in vivo* colchicine treatments.

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