Triploid hemp genetics

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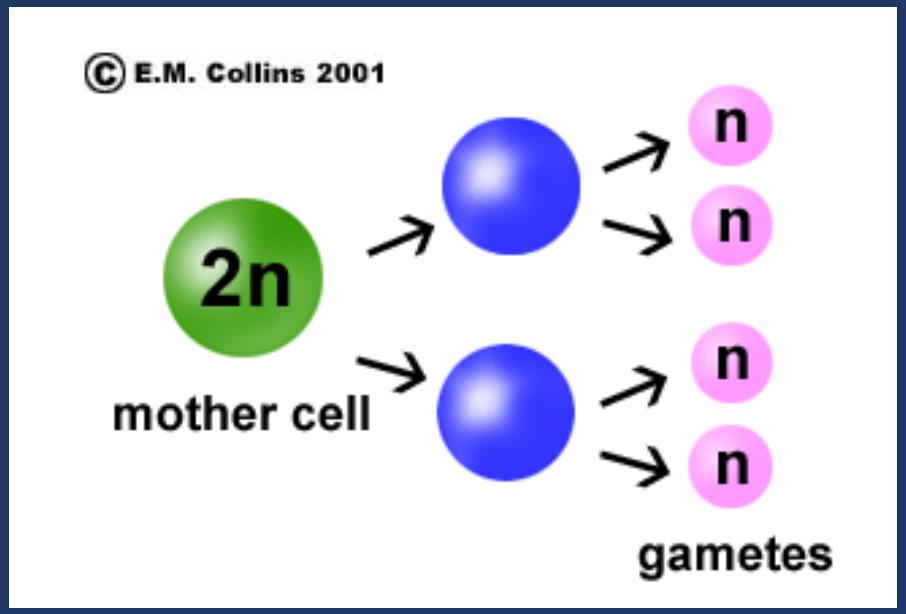
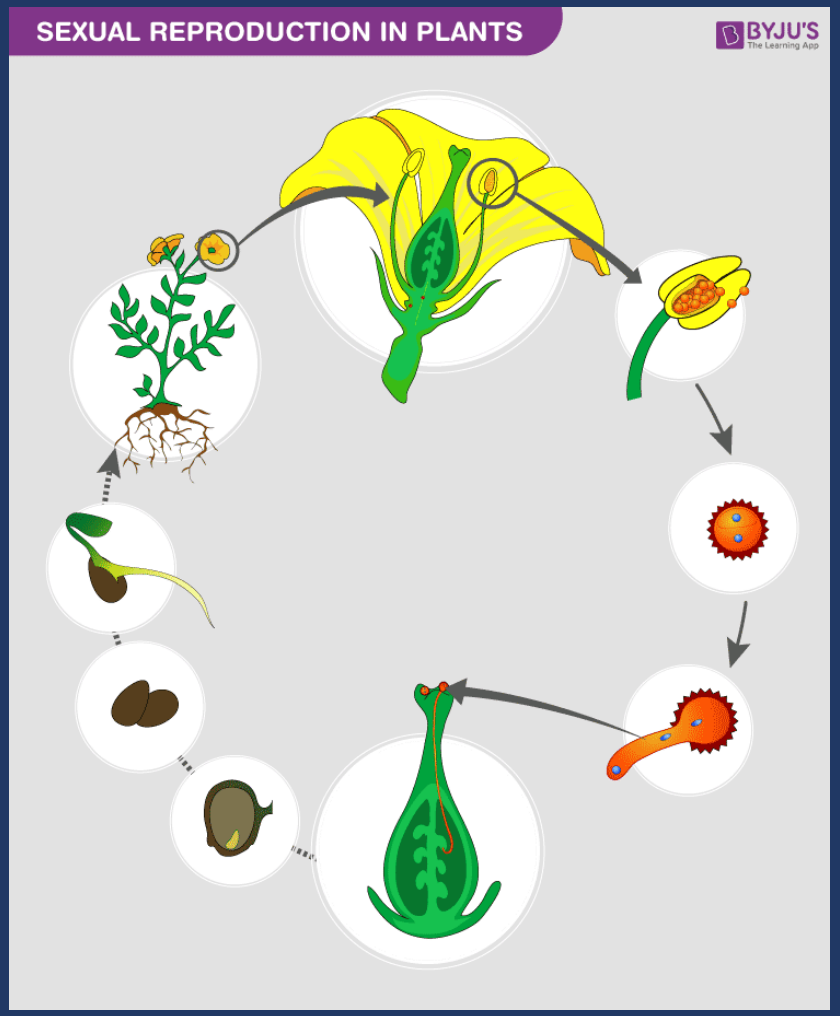
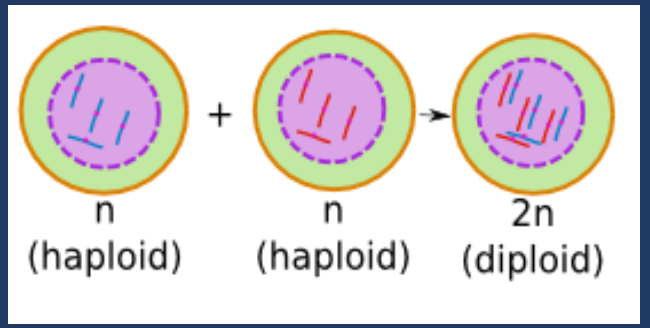
Hemp is naturally diploid (2n)

- Two set of chromosomes (2n)
- Gametes are haploid (1n) and combine to form diploid seed





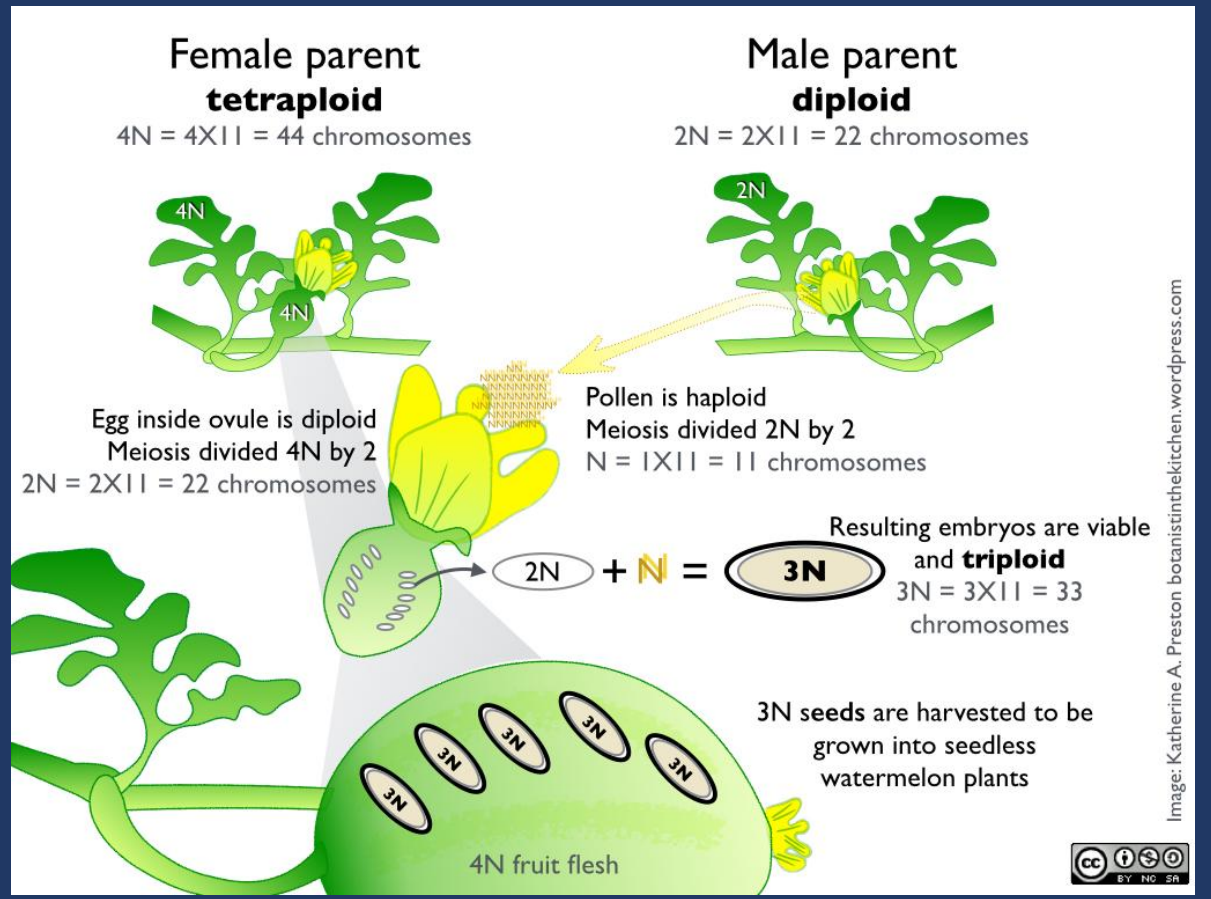


Seed set is problematic for flower production:

- Reduced yield (50%)
- Reduced CBD production (30%)
- Energy diverted to seed
- Seed oil dilutes extract
- Unsalable flower
- Scouting males is labor intensive

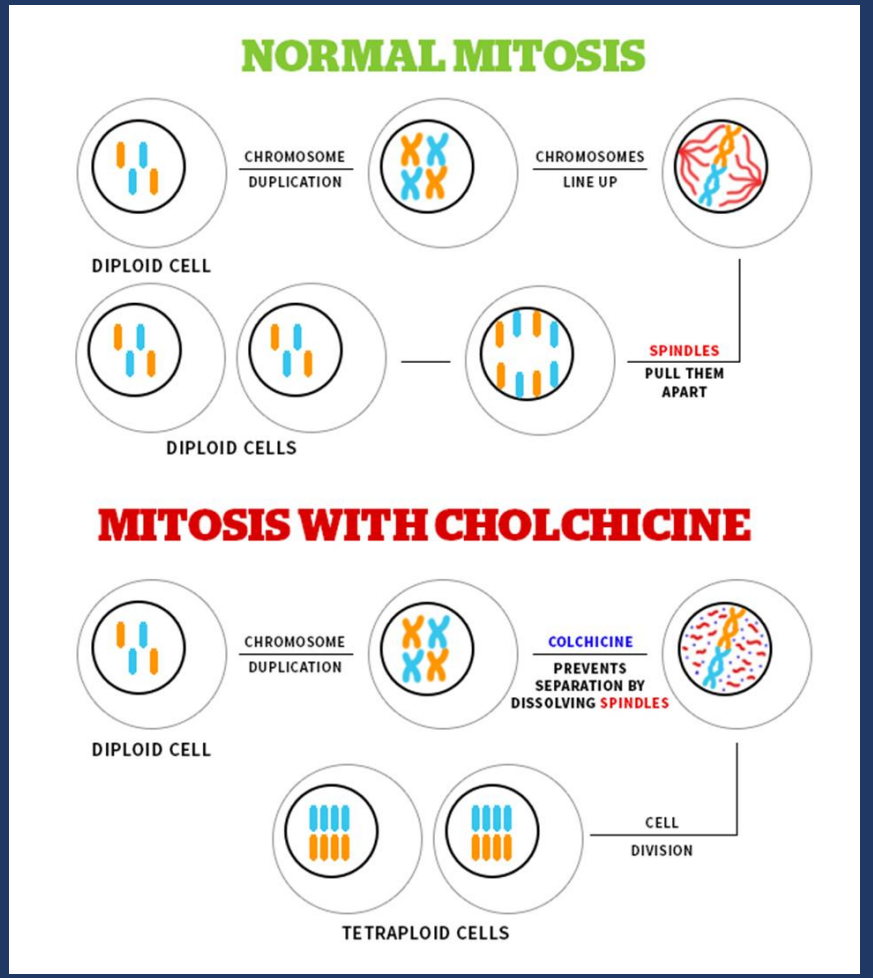
One possible solution is triploid (3n) hemp

- 3 sets of chromosomes
- Triploids produce unviable gametes
- Seed production fails
- Seedless watermelon, citrus, hops
- Not impacted by pollen drift
- To make a triploid (3n) plant, cross a diploid with a tetraploid



First: make a tetraploid hemp plant

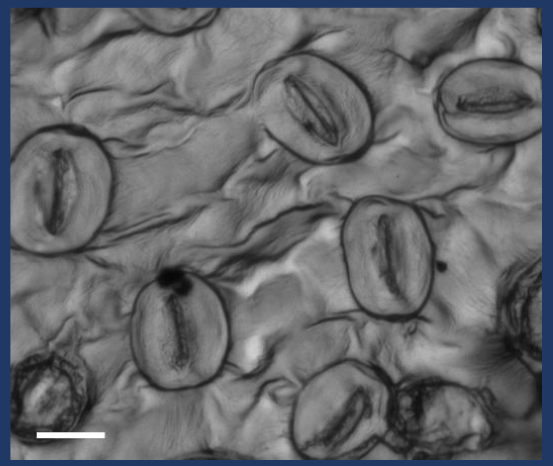
Colchicine disrupts mitosis, doubling chromosomes



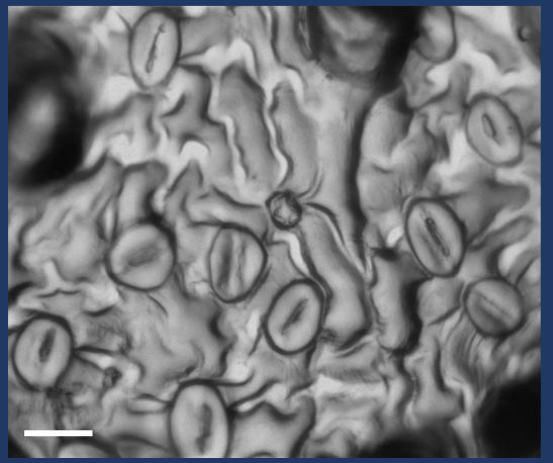
Tetraploid Diploid

Tetraploid Diploid

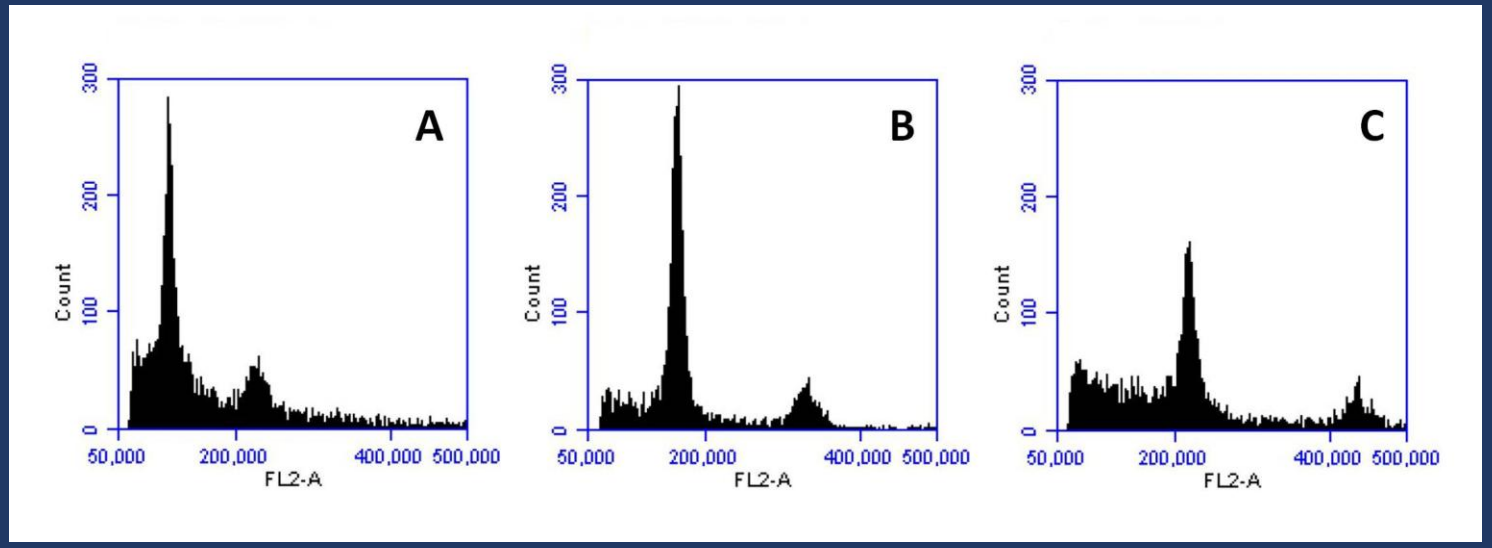
Tetraploid

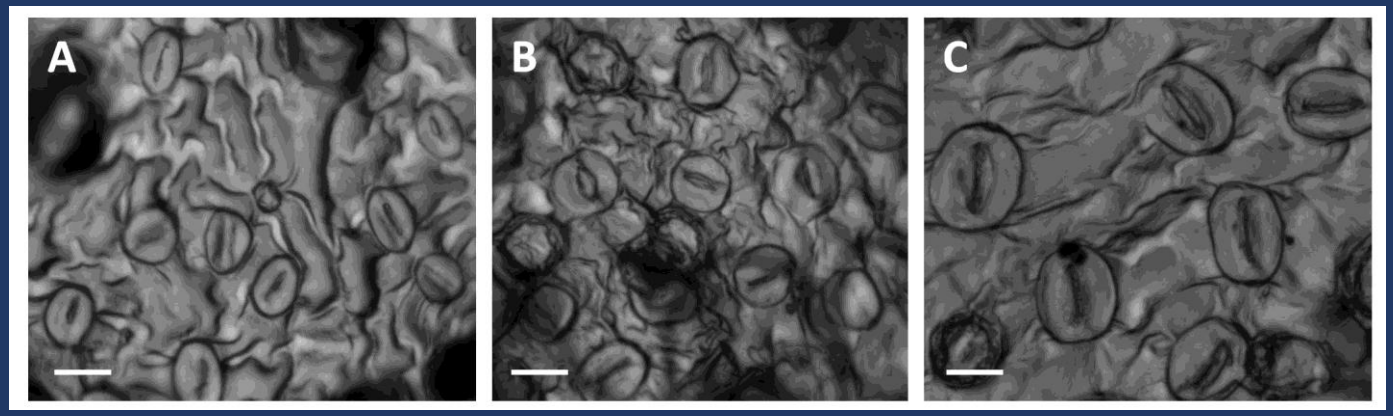


Diploid



Diploid Triploid Tetraploid





Production of Tetraploid and Triploid Hemp

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Abstract. **To maximize yield, cannabidiol (CBD) hemp producers prefer female plants, and this is accomplished by using expensive feminized seed, vegetatively propagated female clones, or by removing male plants from dioecious seed lots. Hemp pollen drifts long distances on wind, and pollination of females reduces CBD content. Induction of triploidy is a common strategy used by plant breeders to produce sterile cultivars of agricultural crops. Triploid (3n) hemp, with three sets of chromosomes, was developed by crossing naturally diploid (2n) hemp with tetraploid (4n) hemp. Tetraploid plants used to create triploids were produced using pregerminated seeds and the mitotic spindle inhibitor colchicine. Seedlings from seeds of 'Abacas' × [('Otto2' × 'BaOx') × ('BaOx' × 'Colorado Cherry')] treated with 0.05% colchicine or 0.02% colchicine for 12 hours and longer were significantly shorter than controls and ≤1 cm tall at 10 days after sowing. Surviving seedlings exhibited thickened cotyledons and hypocotyls, which indicated a potential change in ploidy. Tetraploid induction ranged from 26% to 64% for pregerminated seeds of five different hemp cultivars (Abacus × Wife, Cherry Wine, Mountain Mango, Wife, and Youngsim10) treated with 0.05% colchicine for 12 hours. Tetraploids had nearly twice the DNA content as diploids according to flow cytometric analysis. Tetraploid 'Wife' had larger stomates and reduced stomatal density compared with diploid 'Wife'. Four triploid 'Wife' genotypes produced from crossing tetraploid 'Wife' with diploid 'Wife' were acclimated to greenhouse conditions after embryo rescue. DNA content and stomate size of triploid 'Wife' was intermediate between the parents. This is the first report of triploid plants of hemp. Future research will evaluate the sterility of triploid hemp.**

Cannabis sativa (hemp, marijuana) is a dioecious species with homogametic (XX) pistillate female plants and heterogametic (XY) staminate male plants (Moliterni et al., 2004). The species is cultivated for cannabinoids, most notably CBD and tetrahydrocannabinol (THC), fiber, and grain, from which a wide range of consumer products are derived (Small, 2015). Cannabinoids have reported medicinal value and are produced in the glandular trichomes of the plant, which are found in the greatest density on the inflorescences of female plants (Small and Cronquist, 1976). Hemp is distinguished from marijuana by the content of THC produced by the plant, which is less than 0.3% dry weight THC for hemp (Agriculture Marketing Service, 2019). Hemp fiber is produced from the stalks and the seed is harvested for grain and hempseed oil (Small, 2015). Monecious cultivars have been developed for dual-purpose fiber and grain production.

Hemp seed from open-pollinated dioecious plants can be expected to produce a 50:50 ratio of male-to-female plants (Small, 2015). During CBD hemp production, it is important for growers to remove male plants before anthesis, because pollination of female plants reduces cannabinoid yield (Meier and Mediavilla, 1998). Hemp growers prefer to use feminized seed or vegetatively propagated female clones for CBD production to eliminate the labor of removing male plants and the lost acreage from removed male plants. Hemp is wind-pollinated, and pollen can drift long distances (Small, 2015). It has been reported that hemp pollen can drift more than 300 km (Clarke, 1977). Therefore, even when hemp farmers take strict measures to grow only female plants, they can experience seed production as a result of drifting pollen from neighboring fiber and grain farms or from CBD farms that did not remove males. A distance of at least 5 km is recommended to prevent pollen drift from neighboring hemp fields (Neiden, 2020; Small, 2015). Disputes between farmers over unintended seed production from drifting pollen has led to several lawsuits (Perkowski, 2019). Pollen can also drift from wild or escaped hemp, known as ditch weed (Neiden, 2020).

Induction of polyploidy has been used by plant breeders to develop improved horticultural crops with enhanced traits such as size, vigor, and metabolite content (Alexander, 2017; Lehrer et al., 2008; Sattler et al., 2016; Wang et al., 2016; Xu et al., 2014). Tetraploids are polyploids that contain four sets of chromosomes. Compared with diploids, tetraploid purple cone flower produces more secondary metabolites and biomass (Xu et al., 2014), and tetraploid ryegrass is more drought tolerant and disease resistant (Sattler et al., 2016). Tetraploidy can be induced artificially using mitotic spindle inhibitors such as colchicine or oryzalin (Sattler et al., 2016; Wang et al., 2016). *C. sativa* is almost exclusively diploid (2n = 20) in the wild (Small and Cronquist, 1976). There is only one report of a natural tetraploid of *C. sativa*, from India (Sharma et al., 2015). Tetraploid *C. sativa* has been produced using colchicine on seedling shoot tips (Bagheri and Mansouri, 2015; Mansouri and Bagheri, 2017), and by using oryzalin on in vitro nodal explants (Parsons et al., 2019). Tetraploid plants produced in these studies exhibited traits such as larger leaves and greater shoot fresh weight and flavonoid content.

Tetraploid plants crossed with diploid plants can generate triploid plants, which have three sets of chromosomes (Wang et al., 2016). Triploid plants are frequently seedless, because unequal segregation of chromosome pairs during meiosis results in inviable gametes (Wang et al., 2016). Seedless triploid cultivars have been bred for hops, watermelon, banana, and citrus (Trojak-Goluch and Skomra, 2018; Wang et al., 2016). Warmke and Davidson (1944) reported crossing tetraploid and diploid marijuana and producing triploid plants; however, no cytogenetic evidence of triploidy was provided. The objective of this work was to investigate a more efficient and easy method for inducing tetraploidy in hemp, and to cross tetraploid plants with diploid plants to produce triploid hemp. Triploid hemp that does not produce seed when exposed to pollen could be a solution for the problem of pollen drift.

Materials and Methods

Tetraploid development. Two experiments were conducted to produce tetraploid hemp plants by treating pregerminated seeds with colchicine. In Expt. 1, pregerminated seeds of 'Abacas' × [('Otto2' × 'BaOx') × ('BaOx' × 'Colorado Cherry')] were exposed to two colchicine concentrations (0.02% or 0.05%) for three durations (6, 12, or 24 h) to determine a suitable exposure rate for tetraploid induction. Control pregerminated seeds were treated with water for 24 h. In Expt. 2, pregerminated seeds of five different hemp cultivars (Abacus × Wife, Cherry Wine, Mountain Mango, Wife, and Youngsim10) were treated with 0.05% colchicine for 12 h to generate additional tetraploid genotypes. Seeds were pregerminated by soaking them in water for 24 h and then transferring them to 100- × 15-mm petri dishes lined with moistened filter paper (Whatman no. 4; Whatman, Maidstone, UK) for another 24 h. After this treatment, seeds were considered pregerminated because radicals had emerged from 1 to 5 mm (Fig. 1A). For the colchicine treatment,

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Sterility experiment results

Triploids had ½ the number of stems per plant and total stem length, and similar percent CBD and THC.

Sterility experiment results

Diploid

Avg. 3000 seed/plant

88% brown seed

Triploid

Avg. 100 seed/plant

8% brown seed

Sterility experiment results

Seed viability may depend on:

-maternal:paternal ploidy contribution to *endosperm*

-embryo:endosperm ploidy