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Gene Dosage at the Autoflowering Locus Effects Flowering Timing and Plant Height in Triploid Cannabis

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Abstract. There is demand for early-flowering cannabis (*Cannabis sativa*) cultivars to hasten harvest and avoid late-season detrimental weather conditions. A field study and greenhouse studies were conducted to evaluate the effect of gene dosage at the autoflowering locus on flowering timing for diploid and triploid hybrids between autoflowering and photoperiod-sensitive parents. Autoflowering × photoperiod-sensitive hybrids were all photoperiod sensitive, but their critical photoperiods were longer than for homozygous photoperiod-sensitive plants, which resulted in earlier flowering. For triploid genotypes, decreasing dosage of the photoperiod-sensitive allele (A), from AAA to AAa to Aaa, reduced the time to flowering. Flowering timing for the diploid genotype Aa was intermediate between Aaa and AAa. These results provide evidence of incomplete dominance of the A allele at the autoflowering locus. Plants of genotype Aaa flowered 32 to 40 days earlier in the field than genotypes of AA, 15 days earlier than genotype Aa, and were ready for harvest by the second week of August in Connecticut. Plants of Aaa were as tall as other diploid and triploid photoperiod-sensitive genotypes studied, which suggests that they have similar yield potential. The use of tetraploid autoflowering (aaaa) maternal plants in combination with diploid photoperiod-sensitive (AA) pollen parents to produce Aaa genotype seed is a reliable approach for developing early-flowering cultivars of cannabis for flower production purposes.

Cannabis (*C. sativa*) is a diploid (2x = 20), dioecious species that is grown for fiber, grain, and flower (Ren et al. 2021). Female plants are grown for flower production, because their inflorescences produce the highest concentrations of cannabinoids. Cannabis is predominantly a photoperiod-sensitive, quantitative short-day plant (Petit et al. 2020). The critical photoperiod varies by cultivar, but is typically between 12 and 14 h (Zhang et al. 2021). Photoperiod-insensitive (day-neutral or autoflowering) cultivars of cannabis are available, which initiate flowering based on maturity instead of daylength. The autoflowering trait likely originated from plants growing in northern latitude locations where they experience long days and a short growing season. It has been suggested that autoflowering cannabis plants comprise a separate species or subspecies called *Cannabis ruderalis* or *C. sativa* ssp. *ruderalis* (McPartland 2018; Toth et al. 2022; Zhang et al. 2018). Autoflowering cultivars are used for grain production; however, there is also interest in them for flower production, because they mature significantly faster than photoperiod-sensitive cultivars do (Stack et al. 2021).

Outdoor flower production of photoperiod-sensitive plants requires 3.0 to 4.5 months from planting to harvest, depending on the cultivar and the planting location (Darby et al. 2021a). In northern regions of the United States, planting typically occurs in early to mid-June, which places harvest in late September to late October (Darby et al. 2021a; Stack et al. 2021). At time of harvest, flower yield and quality may be adversely affected by frost and cold, rainy, or humid weather. As a result, moisture-related diseases, including *Fusarium* and *Botrytis,* are a problem for late-to-harvest crops (Darby et al. 2021a). Autoflowering plants offer the benefit of quicker harvest (45 to 75 d), but they yield half as much flower per unit area as photoperiod-sensitive plants (Coolong et al. 2023). It may be possible to complete two crop cycles per growing season using autoflowering plants; however, the second crop will be affected by the same problems that affect late-harvest photoperiod-sensitive plants. Another limitation of autoflowering plants is precocious flowering in response to transplant stress, which can significantly reduce yield potential.

The development of high-yielding, early-flowering cultivars would benefit flower producers, especially in northern latitudes (Stack et al. 2021). Hybrids between autoflowering and photoperiod-sensitive cultivars have been observed to mature slightly faster (~2 weeks) than cultivars homozygous for photoperiod sensitivity at the autoflowering locus, when they are planted in spring in the northeastern United States (Toth et al. 2022). The autoflowering trait has been reported to be homozygous recessive and controlled by a single genetic locus (Green 2005). Working with cannabis cultivars selected for cannabidiol (CBD) content, Toth et al. (2022) identified the locus controlling the autoflowering trait (*autoflower1*) and a separate locus affecting flowering timing, both of which occur on chromosome 1.

Recently, triploid cannabis cultivars, that exhibit significant reduction in fertility, have been developed as a solution for crop loss from seed set during outdoor flower production in areas experiencing pollen drift (Crawford et al. 2021). Triploid plants are frequently seedless, because unequal segregation of chromosome pairs during meiosis results in inviable gametes (Wang et al. 2016). The triploid cannabis cultivar Stem Cell CBG was found to produce 98% less seed than its diploid counterpart, following exposure to feminized pollen (Crawford et al. 2021), which has lower viability than pollen from genetically male

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plants (DiMatteo et al. 2020). If triploid cannabis continues to demonstrate significantly reduced seed set compared with diploids when exposed to pollen, then triploid cultivars will be sought for flower production.

Gene dosage has been shown to affect phenotype in polyploids of various plant species (Baduel et al. 2018; DeMaggio and Lambrukos 1974; Dunn and Namm 1970; Guo et al. 1996; Levin et al. 1979; Serce and Hancock 2005). For example, triploid and tetraploid plants of corn (*Zea mays*) homozygous at the locus for chlorotic lesion resistance exhibited greater disease resistance than homozygous monoploid or diploid plants (Dunn and Namm 1970). The objective of this research was to evaluate how gene dosage at the autoflowering locus in triploids and diploids affects flowering timing of cannabis.

Materials and Methods

Plant material. Plant material of CBD-dominant cannabis photoperiod-sensitive cultivars Abacus, Kentucky Sunshine, and Wife and autoflowering cultivars Purple Star, Tsunami, and Wilhelmina were obtained as seed from reputable commercial sources. Tetraploid plants to serve as parents in controlled crosses were developed for all cultivars except Abacus, and ploidy level was confirmed using flow cytometry, according to the methods in Kurtz et al. (2020). Crosses were conducted in a greenhouse or growth chamber with photoperiod control during 2021 and 2022 and using only genetically female parent plants to produce feminized seed. The designated pollen parent was masculinized using the protocol by Lubell and Brand (2018). Parents of crosses were selected to provide seedling populations that varied in ploidy level and gene dosage at the autoflowering locus, denoted as "a" for autoflowering allele and "A" for photoperiod-sensitive allele. Ploidy level for all experimental plants was confirmed by flow cytometry.

Greenhouse study 1. Seed populations from seven different crosses were evaluated in this study (Table 1), which was conducted from 28 Sep to 30 Dec 2021. Seeds were germinated and seedlings grown in 7.6-L containers with peat-based medium (Metro-Mix 830; Sun Gro Horticulture, Agawam, MA, USA). Containers were top-dressed with 32 g of 15N–3.9P–10K controlled-release fertilizer [CRF (Osmocote Plus 5- to 6-month formulation; Everris NA, Dublin, OH, USA)]. Plants were grown in a greenhouse with set points of 21/17 °C day/night temperature threshold. The experimental unit (EU) was a single potted plant, and plants were arranged in a randomized complete block design (RCBD) with six replications. Plants were fertigated with a 20N–8.7P–16.6K water-soluble fertilizer (Peters 20–20–20; Scotts, Marysville, OH, USA), providing 100 $mg \cdot L^{-1}$ N at each watering. Photoperiod was controlled with supplemental lighting using 1000-W high-pressure sodium (HPS) lamps (Phantom HPS 100W; Hydrofarm, Petaluma, CA, USA) and blackout curtains. Plants were grown vegetatively under 18-h photoperiod for the first 34 d of the study. The photoperiod was reduced to 15 h for days 35 to 55 of the study, and reduced again to 12 h from days 56 to 92 of the study. Plants were observed daily for initiation of terminal flowering, which was determined when a minimum of three pistils bearing stigmas were visible at the shoot tips, according to Spitzer-Rimon et al. (2019).

Greenhouse study 2. Seed populations from seven different crosses were evaluated in this study (Table 1), which was conducted from 25 Apr to 22 Jul 2022. Seedlings were grown in 11.4-L containers and top-dressed with 52 g of CRF as described. The EU was a single potted plant and plants were arranged as an RCBD with six replications. All other cultivation and data collection methods were as described for greenhouse study 1, except for the photoperiod conditions. Plants were allowed to grow vegetatively under 18-h photoperiod for the first 28 d of the study. Then the photoperiod was reduced to 15 h for 7 d, then 14.5 h for 7 d, then 14 h for 7 d, then 13.5 h for 7 d, then 13 h for 7 d, then 12.5 h for 7 d, then 12 h for 14 d, at which time the study was completed.

Field study. Seedling populations from nine different crosses were evaluated (Table 2). Seeds were germinated on 6 May 2022 and seedlings grown in 307-mL containers in a greenhouse under 18-h photoperiod until transplanting to the field on 31 May 2022. The field planting was located at the University of Connecticut Plant Science Research and Education Facility in Storrs, CT, USA (lat. 41.79544°N, long. 72.22836°W). The field soil was a Paxton and Montauk fine sandy loam with 6.7% organic matter and pH 5.9. Plants were grown in rows with 1.8-m spacing on center between rows and 1.2-m spacing on center within rows. The EU was a single plant and plants were arranged as an RCBD with 10 replications (90 plants total). There were two blocks per planting row for a total of five rows. Plants were fertilized twice, at time of planting and again on 7 Jul 2022, with 10 g of granular fertilizer (All Purpose 10N–4.4P–8.3K; Greenview, Lebanon, PA, USA) per plant each time. Fertilizer was broadcast around the base of the plant by hand. Plants were irrigated by hand as needed throughout the growing season. Weeding was by rototiller between rows and by hand within rows.

Table 1. Cannabis (*Cannabis sativa*) parental cross (autoflowering locus genotype, where a = autoflowering allele and A = photoperiod-sensitive allele), seedling ploidy, seedling genotype at autoflower/photoperiod-sensitive locus, and greenhouse study in which the seedling population was included.

Parental cross (pistillate × staminate)	Seedling ploidy	Seedling genotype at autoflowering/ photoperiod-sensitive locus	Greenhouse study no.
'Purple Star' (aaaa) × 'Wilhelmina' (aa)	3x	aaa	2
'Purple Star' (aaaa) × 'Purple Star' (aa)	3x	aaa	1
'Purple Star' (aa) × 'Purple Star' (aa)	2x	aa	1
'Purple Star' (aa) × 'Wife' (AA)	2x	Aa	1 and 2
'Tsunami' (aaaa) × 'Wife' (AA)	3x	Aaa	2
'Purple Star' (aaaa) × 'Wife' (AA)	3x	Aaa	1 and 2
'Wife' (AAAA) × 'Purple Star' (aa)	3x	AAa	1 and 2
'Wife' (AA) × 'Wife' (AA)	2x	AA	1 and 2
'Kentucky Sunshine' (AAAA) × 'Wife' (AA)	3x	AAA	1 and 2

Table 2. Cannabis (*Cannabis sativa*) parental cross (autoflowering locus genotype, where a = autoflowering allele and A = photoperiod-sensitive allele), seedling ploidy, seedling genotype at autoflower/photoperiod-sensitive locus, and plant height for plants grown in the field study.

Parental cross (pistillate × staminate)	Seedling ploidy	Seedling genotype at autoflowering/ photoperiod-sensitive locus	Plant ht (cm)
'Purple Star' (aaaa) × 'Wilhelmina' (aa)	3x	aaa	44 e[i]
'Tsunami' (aa) × 'Wilhelmina' (aa)	2x	aa	53 e
'Tsunami' (aaaa) × 'Wife' (AA)	3x	Aaa	179 cb
'Purple Star' (aaaa) × 'Wife' (AA)	3x	Aaa	160 d
'Purple Star' (aa) × 'Wife' (AA)	2x	Aa	170 cd
'Wife' (AAAA) × 'Purple Star' (aa)	3x	AAa	180 cb
'Abacus' (AA) × 'Wife' (AA)	2x	AA	206 a
'Wife' (AA) × 'Wife' (AA)	2x	AA	187 b
'Kentucky Sunshine' (AAAA) × 'Wife' (AA)	3x	AAA	202 a

[i] Mean separation indicated by different letters using Fisher's least significant difference at $P \leq 0.05$ (n = 10).

Plants were observed weekly for initiation of terminal flowering, which was determined as described for greenhouse studies. For each population, peak flowering was noted when terminal colas had appeared to reach their maximum size and trichomes began changing from clear to translucent white. Plant height, measured from base to tip, was recorded for autoflowering plants on 2 Aug 2022, and all other plants on 1 Sep 2022.

Statistical analysis. Data were subjected to analysis of variance (PROC GLIMMIX) and mean separation by Fisher's least significant difference test ($P \leq 0.05$) using statistical software (SAS version 9.4; SAS Institute, Cary, NC, USA).

Results

In greenhouse study 1, number of days to terminal flowering was significantly different for all genotypes, except aa and aaa were similar to each other (Fig. 1). Number of days to terminal flowering progressively increased according to the following order of genotypes: aa/aaa, Aaa, Aa, AAa, AA, AAA. In greenhouse study 2, number of days to terminal flowering was greatest for the AA and AAA genotypes and the lowest for aaa (Fig. 2). Days to terminal flowering for the remaining genotypes were intermediate between aaa and AA/AAA. Both Aaa genotypes flowered at a similar time and sooner than genotypes Aa and AAa, which were not significantly different from each other.

In the field study, timing of terminal flowering was similar for the autoflowering genotypes aa and aaa (Fig. 3). Also, terminal flowering did not differ between the two Aaa genotypes. Flowering timing differed significantly among all other genotypes, which also differed from Aaa and autoflowering genotypes aa and aaa. Genotypes AAA and AA ('Abacus' × 'Wife') were the tallest and the autoflowering genotypes aa and aaa were the shortest (Table 2). All other genotypes were intermediate in height and more than 200% taller than the autoflowering genotypes. Presence of the a allele, when mixed with A alleles, produced plants that were slightly shorter than homozygous photoperiod-sensitive hybrid genotypes. Genotype Aaa 'Tsunami' × 'Wife' was taller than genotype Aaa 'Purple Star' × 'Wife', likely due to genetic influence of the maternal parent. Genotype Aaa 'Purple Star' × 'Wife' was shorter than genotype AAa 'Wife' × 'Purple Star', likely due to gene dosage differences at the autoflowering locus. The diploid Aa had similar height as Aaa and AAa triploids.

Discussion

This research corroborates the reports of others that the autoflowering trait in CBD-dominant cannabis is controlled by a single locus and is homozygous recessive (Green 2005; Toth et al. 2022). Hybrids between photoperiod-sensitive and autoflowering

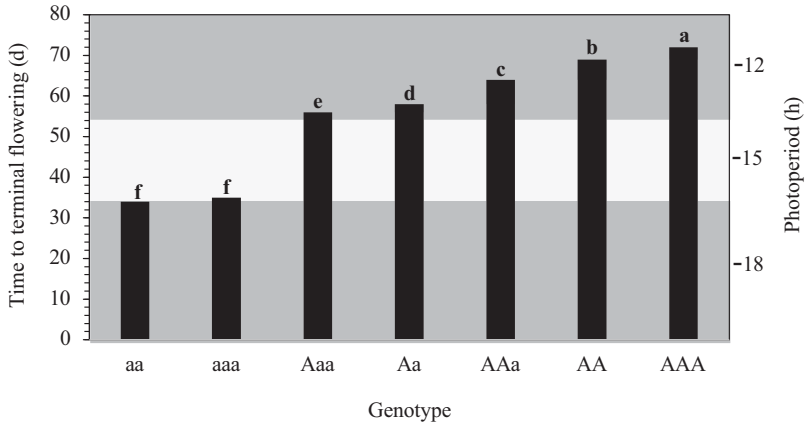

Fig. 1. Days to terminal flowering for cannabis (*Cannabis sativa*) plants grown in a greenhouse for 34 d at 18-h photoperiod, followed by 20 d at 15-h photoperiod, followed by 36 d at 12-h photoperiod. Shaded horizontal bars indicate different photoperiods. Mean separation indicated by different letters, using Fisher's least significant difference test at $P \leq 0.05$ (n = 6). aa = 'Purple Star' (aa) × 'Purple Star' (aa); aaa = 'Purple Star' (aaaa) × 'Purple Star' (aa); Aaa = 'Purple Star' (aaaa) × 'Wife' (AA); Aa = 'Purple Star' (aa) × 'Wife' (AA); AAa = 'Wife' (AAAA) × 'Purple Star' (aa); AA = 'Wife' (AA) × 'Wife' (AA); AAA = 'Kentucky Sunshine' (AAAA) × 'Wife' (AA).

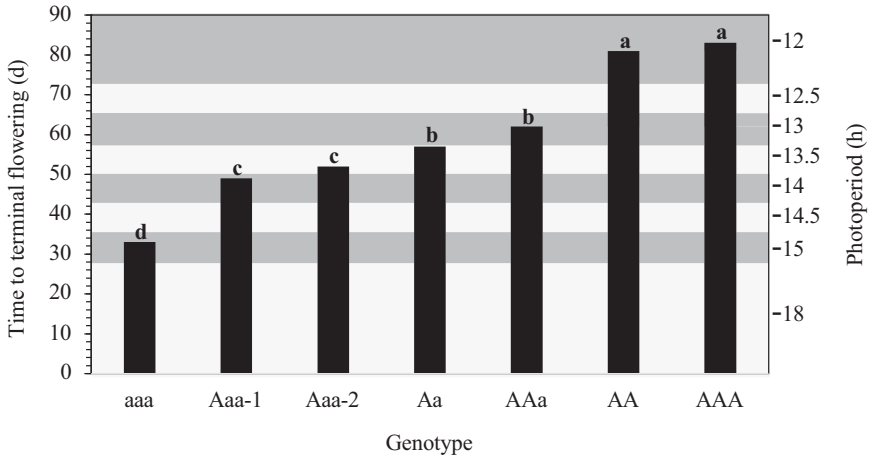

Fig. 2. Days to terminal flowering for cannabis (*Cannabis sativa*) plants grown in a greenhouse under 18-h photoperiod for 28 d, then 15 h for 7 d, then 14.5 h for 7 d, then 14 h for 7 d, then 13.5 h for 7 d, then 13 h for 7 d, then 12.5 h for 7 d, then 12 h for 14 d. Shaded horizontal bars indicate different photoperiods. Mean separation indicated by different letters, using Fisher's least significant difference test at $P \leq 0.05$ (n = 6). aaa = 'Purple Star' (aaaa) × 'Wilhelmina' (aa); Aaa-1 = 'Tsunami' (aaaa) × 'Wife' (AA); Aaa-2 = 'Purple Star' (aaaa) × 'Wife' (AA); Aa = 'Purple Star' (aa) × 'Wife' (AA); AAa = 'Wife' (AAAA) × 'Purple Star' (aa); AA = 'Wife' (AA) × 'Wife' (AA); AAA = 'Kentucky Sunshine' (AAAA) × 'Wife' (AA).

cultivars were found to be photoperiod sensitive; however, their critical photoperiod was significantly longer, resulting in earlier onset of terminal flowering than for genotypes homozygous for photoperiod sensitivity (Figs. 1–3). Therefore, we conclude that the photoperiod-sensitive allele exhibits incomplete dominance at the autoflowering locus. This locus may be more accurately described as the autoflowering/photoperiod-sensitive locus. In jute (*Corchorus olitorius*) and common bean (*Phaseolus vulgaris*), photoperiod sensitivity is also primarily controlled by a single gene locus and is incompletely dominant (Gu et al. 1998; Hossain et al. 2001).

For the triploid genotypes evaluated, dosage of the A allele may be considered equivalent to 33%, 66%, and 100% for genotypes Aaa, AAa, and AAA, respectively. As dosage of the A allele decreased, the time to terminal flowering also decreased, and this could prove to be useful for breeding early-flowering cultivars. During outdoor field production, Aaa genotypes initiated terminal flowering 27 d earlier than AAa, and 45 d earlier than AAA (Fig. 3). Incomplete dominance and gene dosage resulted in two intermediate phenotypes in triploid and tetraploid hybrids of spiderwort [*Tradescantia* (Anderson 1935)]. For five unrelated morphological traits, tetraploid spiderwort hybrids (AABB) were exactly intermediate between the two parents, and triploid hybrids (AAB) were intermediate between the parent that contributed two alleles and the tetraploid hybrid. In our field studies, onset of terminal flowering for the diploid Aa genotype

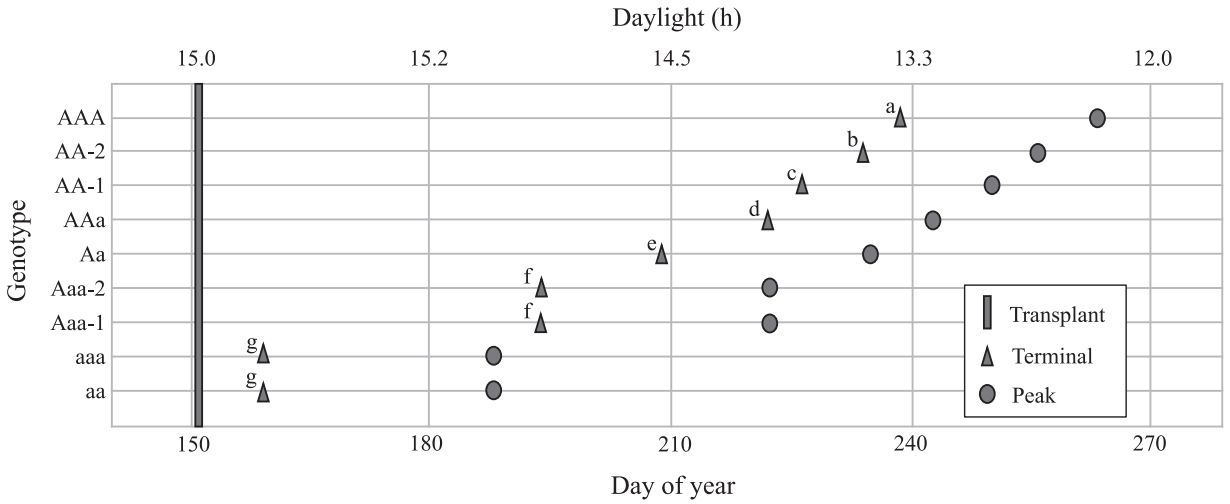

Fig. 3. Timeline of terminal flowering and peak flowering for cannabis (*Cannabis sativa*) genotypes: aa = 'Tsunami' (aa) × 'Wilhelmina' (aa); aaa = 'Purple Star' (aaaa) × 'Wilhelmina' (aa); Aaa-1 = 'Tsunami' (aaaa) × 'Wife' (AA); Aaa-2 = 'Purple Star' (aaaa) × 'Wife' (AA); Aa = 'Purple Star' (aa) × 'Wife' (AA); AAa = 'Wife' (AAAA) × 'Purple Star' (aa); AA-1 = 'Abacus' (AA) × 'Wife' (AA); AA-2 = 'Wife' (AA) × 'Wife' (AA); AAA = 'Kentucky Sunshine' (AAAA) × 'Wife' (AA). The vertical bar indicates transplant date. Triangles indicate terminal flowering and circles indicate peak flowering. Mean separation indicated by different letters, using Fisher's least significant difference test at $P \leq 0.05$ (n = 10).

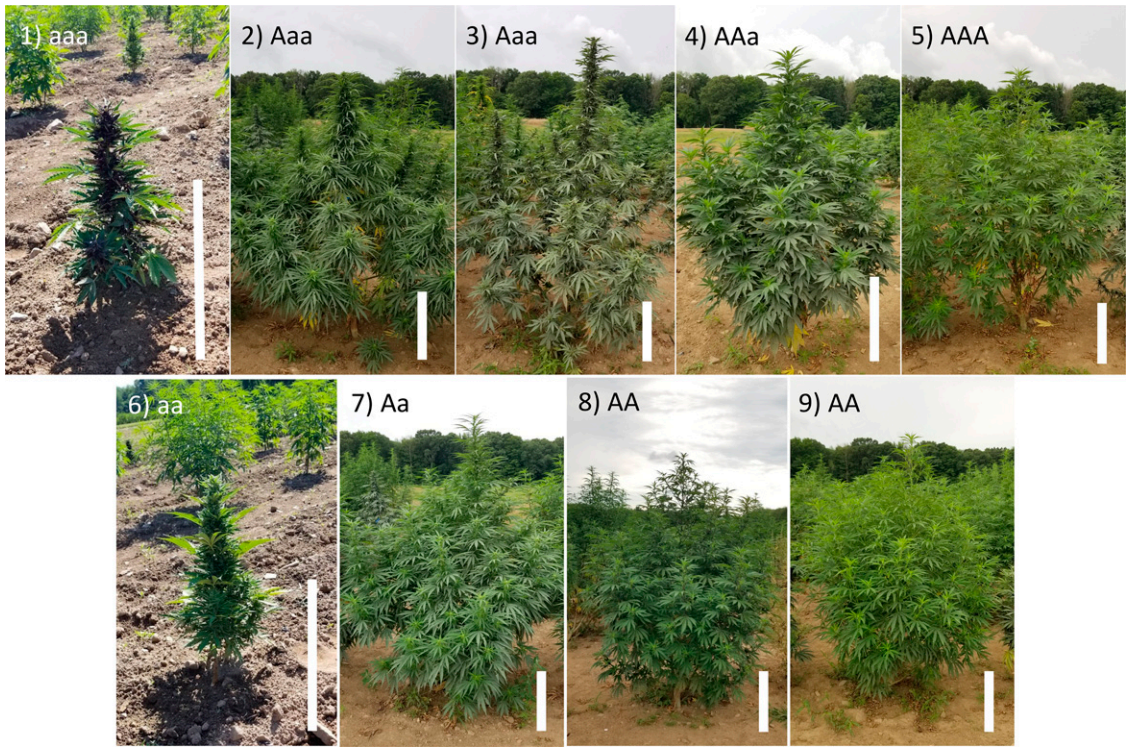

Fig. 4. Cannabis (*Cannabis sativa*) plants of genotypes 1) aaa = 'Purple Star' × 'Wilhelmina', 2) Aaa = 'Tsunami' (aaaa) × 'Wife' (AA), 3) Aaa = 'Purple Star' (aaaa) × 'Wife' (AA), 4) AAa = 'Wife' (AAAA) × 'Purple Star' (aa), 5) AAA = 'Kentucky Sunshine' (AAAA) × 'Wife' (AA), 6) aa = 'Tsunami' × 'Wilhelmina', 7) Aa = 'Purple Star' (aa) × 'Wife' (AA), 8) AA = 'Abacus' (AA) × 'Wife' (AA), and 9) AA = 'Wife' (AA) × 'Wife' (AA) in the field on 30 Jun 2022 (day 181 of the year) for autoflowering genotypes aa and aaa and on 11 Aug 2022 (day 223 of the year) for all other genotypes at the University of Connecticut, Plant Science Research and Education Facility in Storrs, CT, USA; scale bars = 45 cm.

(50% dosage of the A allele) was intermediate between Aaa and AAa, as would be expected for incomplete dominance (Fig. 3). Commercial cultivars that are reported to be diploid hybrids between photoperiod-sensitive and autoflowering parents demonstrate intermediate phenotype for flowering date as well as plant height and wet biomass (Toth et al. 2022).

Triploid and diploid cultivars developed by Oregon CBD (Independence, OR, USA) have been bred using a diploid autoflowering pollen parent and possess the genotypes AAa and Aa at the autoflowering locus (Stack et al. 2022). In field trials at the University of Vermont (Burlington, VT, USA), Oregon CBD triploids flowered 1 to 2 weeks later than their diploid counterparts (Darby et al. 2021b), which agrees with our findings about flowering timing differences for AAa and Aa genotypes. Further, we found AAA initiated flowering 8 to 13 d later than AA genotypes in the field (Fig. 3). Later flowering of autopolyploids compared with related diploids has been reported previously (Husband and Sabara 2004; Levin 1983). For example, tetraploids of fireweed (*Chamerion angustifolium*) were found to flower 8 and 10 d later than diploids in greenhouse and field studies, respectively (Husband and Sabara 2004). In our studies, there was no difference in flowering timing between aaa and aa, probably because the vegetative period of growth for these autoflowering plants was so short (Figs. 1 and 3).

The two genotypes of AA (Table 2) differed in flowering timing by 8 d in the field (Fig. 3). The difference in timing between AA genotypes is likely due to their overall genetics and not the autoflowering locus. This degree of variation in flowering timing is well within the range that has been reported for diploid photoperiod-sensitive cultivars grown for flower (Darby et al. 2021b; Stack et al. 2021; Zhang et al. 2021).

In the field, our genotypes of Aaa initiated terminal flowering 15 d earlier than the Aa diploid and 32 to 40 d earlier than the AA diploids (Fig. 3). Flowering timing was highly uniform for all plants of genotype Aaa (all plants initiating at day 194 of the year) from both parental combinations (Table 2). The Aaa genotypes evaluated are considered early flowering, because they had reached peak flowering and were ready to harvest in early to mid-August (Fig. 3) (Stack et al. 2021). Plants of genotype Aaa were as tall as photoperiod-sensitive diploid genotypes (Table 2; Fig. 4) and would likely match their yield potential. Hybrids between photoperiod-sensitive and autoflowering cultivars were observed to be highly uniform in plant stature, likely owing to heterosis, because their individual parents were quite divergent in phenotype (Small 2015). The use of tetraploid autoflowering maternal plants to produce seed of the genotype Aaa is a route that breeders should consider for developing low-seeding, early-flowering, triploid cultivars of cannabis for flower production purposes. The tetraploid autoflowering maternal genotype may be maintained for breeding purposes as feminized seed by crossing two tetraploid autoflowering female plants.

References Cited

Anderson E. 1935. A morphological comparison of triploid and tetraploid interspecific hybrids in *Tradescantia*. Genetics. 21:61–65. https://doi.org/10.1093/genetics/21.1.61.

Baduel P, Bray S, Vallejo-Marin M, Kolar F, Yant L. 2018. The "polyploid hop": Shifting challenges and opportunities over the

evolutionary lifespan of genome duplications. Front Ecol Evol. 6:117. https://doi.org/10.3389/fevo.2018.00117.
Crawford S, Rojas BM, Crawford E, Otten M, Schoenemberger TA, Garfinkel AR, Chen H. 2021. Characteristics of the diploid, triploid, and tetraploid versions of a cannabigerol-dominant F1 hybrid industrial hemp cultivar, *Cannabis sativa* 'Stem Cell CBG'. Genes (Basel). 12:923. https://doi.org/10.3390/genes12060923.
Coolong T, Cassity-Duffey K, Joy N. 2023. Role of planting date on yield and cannabinoid content of day neutral and photoperiod-sensitive hemp in Georgia, USA. HortTechnology. 33:138–145. https://doi.org/10.21273/HORTTECH05151-22.
Darby H, Bruce J, Sullivan L. 2021a. 2021 Hemp flower harvest date. https://www.uvm.edu/sites/default/files/Northwest-Crops-and-Soils-Program/2021%20Research%20Rpts/2021_Hemp_Flower_Harvest_Datefinal.pdf [accessed 22 Dec 2022].
Darby H, Bruce J, Sullivan L, Zeigler S. 2021b. 2021 Hemp flower variety trial. https://www.uvm.edu/sites/default/files/Northwest-Crops-and-Soils-Program/2021%20Research%20Rpts/2021_UVM_Hemp_Flower_Variety_Trial_Final.pdf [accessed 22 Dec 2022].
DeMaggio AE, Lambrukos J. 1974. Polyploidy and gene dosage effects on peroxidase activity in ferns. Biochem Genet. 12:429–440. https://doi.org/10.1007/BF00486060.
DiMatteo J, Kurtz LE, Lubell-Brand JD. 2020. Pollen appearance and in vitro germination varies for five strains of female hemp masculinized using silver thiosulfate. HortScience. 55:547–549. https://doi.org/10.21273/HORTSCI14842-20.
Dunn GM, Namm T. 1970. Gene dosage effects on monogenic resistance to northern corn leaf blight. Crop Sci. 10:352–354. https://doi.org/10.2135/cropsci1970.0011183X001000040010x.
Green G. 2005. The cannabis breeder's bible: The definitive guide to marijuana genetics, cannabis botany and creating strains for the seed market. Green Candy Press, San Francisco, CA, USA.
Gu W, Zhu J, Wallace DH, Singh SP, Weeden NF. 1998. Analysis of genes controlling photoperiod sensitivity in common bean using DNA markers. Euphytica. 102:125–132. https://doi.org/10.1023/A:1018340514388.
Guo M, Davis D, Birchler JA. 1996. Dosage effects on gene expression in a maize ploidy series. Genetics. 142:1349–1355. https://doi.org/10.1093/genetics/142.4.1349.
Hossain M, Hossain F, Basu AK, Roychoudhury P. 2001. Inheritance of photoperiod sensitivity in jute (*Corchorus olitorius* L.). Indian J Genet. 61:140–142.
Husband BC, Sabara HA. 2004. Reproductive isolation between autotetraploids and their diploid progenitors in fireweed, *Chamerion angustifolium* (Onagraceae). New Phytol. 161:703–713. https://doi.org/10.1046/j.1469-8137.2004.00998.x.
Kurtz LE, Brand MH, Lubell-Brand JD. 2020. Production of tetraploid and triploid hemp. HortScience. 55:1703–1707. https://doi.org/10.21273/HORTSCI15303-20.
Levin DA, Torres AM, Levy M. 1979. Alcohol dehydrogenase activity in diploid and autotetraploid phlox. Biochem Genet. 17:35–42. https://doi.org/10.1007/BF00484472.
Levin D. 1983. Polyploidy and novelty in flowering plants. Am Nat. 122:1–25. https://doi.org/10.1086/284115.
Lubell JD, Brand MH. 2018. Foliar sprays of silver thiosulfate produce male flowers on female hemp plants. HortTechnology. 28:743–747. https://doi.org/10.21273/HORTTECH04188-18.
McPartland JM. 2018. Cannabis systematics at the levels of family, genus, and species. Cannabis Cannabinoid Res. 3:203–212. https://doi.org/10.1089/can.2018.0039.
Petit J, Salentijn EMJ, Paulo MJ, Denneboom C, Trindade LM. 2020. Genetic architecture of flowering time and sex determination in hemp (*Cannabis sativa* L.): A genome wide association study. Front Plant Sci. 11:569958. https://doi.org/10.3389/fpls.2020.569958.
Ren G, Zhang X, Li Y, Ridout K, Serrano-Serrano ML, Yang Y, Liu A, Ravikanth G, Nawaz MA, Mumtaz AS, Salamin N, Fumagalli L. 2021. Large-scale whole-genome resequencing unravels the domestication history of *Cannabis sativa.* Sci Adv. 7:eabg2286. https://doi.org/10.1126/sciadv.abg2286.
Serce S, Hancock J. 2005. Inheritance of day-neutrality in octoploid species of *Fragaria.* J Am Soc Hortic Sci. 130:580–584. https://doi.org/10.21273/jashs.130.4.580.
Small E. 2015. Evolution and classification of *Cannabis sativa* (marijuana, hemp) in relation to human utilization. Bot Rev. 81:189–294. https://doi.org/10.1007/s12229-015-9157-3.
Spitzer-Rimon B, Duchin S, Bernstein N, Kamenetsky R. 2019. Architecture and florogenesis in female *Cannabis sativa* plants. Front Plant Sci. 10:1–11. https://doi.org/10.3389/fpls.2019.00350.
Stack GM, Toth JA, Carlson CH, Cala AR, Marrero-Gonzalez MI, Wilk RL, Genter DR, Crawford JL, Philippe G, Rose JK, Viands DR, Smart CD, Smart LB. 2021. Season long characterization of high cannabinoid hemp reveals variation in cannabinoid accumulation, flowering time, and disease resistance. Glob Change Biol Bioenergy. 13:546–561. https://doi.org/10.1111/gcbb.12793.
Stack G, Murdock M, Wares A, Snyder S, Toth J, Crawford J, Schessel M, Genna N, Phillipe G, Stansell Z, Rose J, Moore G, Smart L. 2022. Triploid pollen-challenge trials. https://hemp.cals.cornell.edu/resources/reports-factsheets/2022-cornell-hemp-field-day-handouts/triploid-pollen-challenge-trials [accessed 12 Dec 2022].
Toth JA, Stack GM, Carlson CH, Smart LB. 2022. Identification and mapping of major-effect flowering time loci Autoflower1 and Early1 in *Cannabis sativa* L. Front Plant Sci. 13:991680. https://doi.org/10.3389/fpls.2022.991680.
Wang X, Cheng ZM, Zhi S, Xu F. 2016. Breeding triploid plants: A review. Czech J Genet Plant Breed. 52:41–52. https://doi.org/10.17221/151/2015-CJGPB.
Zhang M, Anderson SL, Brym ZT, Pearson BJ. 2021. Photoperiodic flowering response of essential oil, grain, and fiber hemp cultivars. Front Plant Sci. 12:694153. https://doi.org/10.3389/fpls.2021.694153.
Zhang Q, Chen X, Guo H, Trindade LM, Salentijn EMJ, Guo R, Guo M, Xu Y, Yang M. 2018. Latitudinal adaptation and genetic insights into the origins of *Cannabis sativa* L. Front Plant Sci. 9:1876. https://doi.org/10.3389/fpls.2018.01876.