

Research Paper

Ploidy variation and agronomic performance of F₁ hybrids of tetraploid and diploid forms of *Humulus lupulus* L.

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Humulus lupulus ($2n = 2x = 20$) as a source of hop resins, essential oils and polyphenols has value in brewing, pharmacy and cosmetology. Conventional crossing between tetraploids of ‘Sybilla’ and diploid males were performed to obtain F₁ hybrids. Cytological studies revealed that 83.8% of the hybrids were triploids ($2n = 3x = 30$), 15.2% were aneuploids in which the chromosome number ranged from 28–32. Tetraploids ($2n = 4x = 40$) and diploids were also observed, which indicates numerous disturbances of gametogenesis of the parental forms. STS markers specific for male plants showed that females outnumbered male individuals among the F₁ hybrids, which is in accordance with the distribution of sex ratio characteristic for diploid hybrids of *H. lupulus*. Female triploids were compared to the control ‘Sybilla’ with regard to their functional characteristics and alpha acids content in cones. A two year-long experiment showed that most of the triploids had a significantly higher position of fructiferous branches and shoot twist index compared to diploids of ‘Sybilla’. There was also a significantly extended time for them to reach technological maturity of cones. Triploids were distinguished by a significantly lower seed content compared to ‘Sybilla’, therefore the raw material obtained from them is more suitable for the production of hop pellets and extracts.

Key Words: *Humulus lupulus*, STS markers, sex, triploid, aneuploid, seedlessness.

Introduction

The production of polyploids is a widely used method for crop quality improvement. Duplication of the entire genome frequently contributes to an increase in plant yield or better adaptation to habitat conditions. It is estimated that about 70% of flower plants are polyploids. These are usually autopolyploids, whose chromosomes constitute multiples of the basic number of chromosomes of one species and allopolyploids or auto-allopolyploids, which are the result of interspecies crossing and doubling of the number of chromosomes in parental genomes (Otto 2007). There are also species for which the presence of three haploid genomes can positively affect certain functional traits. These include among others *Miscanthus giganteus* (Jeżowski *et al.* 2007), *Malus domestica* (Janick *et al.* 1996) and *Humulus lupulus* (Beatson and Alspach 2007).

H. lupulus L. ($2n = 2x = 20$) is a wind-pollinated perennial of the Cannabaceae family cultivated mainly for the brewing industry. The specific secondary metabolites contained in the cones give beer its bitter taste and characteris-

tic aroma. Hop polyphenols such as xanthohumol and desmethyl xanthohumol make it suitable for pharmaceutical, medical and cosmetic applications (Stevens and Page 2004). Hop is a dioecious species, distinguished by a heteromorphic sex chromosomes, where females have XX chromosomes and males XY. The sex of hop offspring is determined by the ratio of the number of X chromosomes to the number of sets of autosomes (X/A). A X/autosome ratio of ≤ 0.5 indicates male individuals, while a value of 1 characterizes female plants (Grabowska-Joachimciak *et al.* 2006).

Attempts to grow triploid hops ($2n = 3x = 30$) were made in the United States as early as in the 1970s (Haunold 1971). The majority of triploid cultivars were obtained by controlled or free pollination of tetraploid female forms with diploid male pollen $4x \times 2x$ (Beatson *et al.* 2003, Beatson and Alspach 2007, Probasco *et al.* 2006). A few were obtained in offspring from free pollination of triploid forms with diploid pollen $3x \times 2x$ (Beatson and Alspach 2007). Additionally, the triploid hop plant ‘3/19’ has been obtained by crossing the diploid cultivar ‘Talisman’ with the diploid male ‘5/23’ ($2x \times 2x$) (Grabowska-Joachimciak *et al.* 2006). All of these authors report that triploids are characterized by more intensive growth compared to diploids. Haunold (1972) showed that the daily shoot growths of triploids of ‘Fuggle’ were up to 18 mm longer than of

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those of diploids. Krajl (1973), studying triploid plants obtained on the basis of 'Atlas', recorded a significantly higher number of lupulin glands than in the diploid maternal form. Additionally, author observed an increase in the content of soft resins in triploid cones together with an increase in the size of their lupulin glands. Probasco *et al.* (2006) stated that the alpha acids content in the cones of the triploid cultivar 'Millenium' was 2.5% higher than that of the maternal diploid cultivar 'Nugget'. Similarly, the concentration of certain components of essential oils, such as humulene and carriophilene, which give beer its characteristic aroma, was also higher. According to Beatson *et al.* (2003), the degree of ploidy of hops modifies the yield potential of plants. The triploid 'Nelson Sauvín' showed a higher yield than the diploid commercial cultivars 'Green Bullet' and 'Nugget'. Probasco *et al.* (2006) reported that the triploid cultivar 'Millenium' had a yield higher by 560.7 kg than the diploid 'Nugget'. As far as we know there are no reports regarding an assessment of the morphological traits that characterize triploids.

Another characteristic that triploids can possess is seedlessness. The presence of three sets of chromosomes prevents the proper course of meiosis and the distribution of chromosomes to progeny cells in balanced proportions. Aneuploid gametes are formed, hence triploids show low fertility and low seed-setting capacity (Zhang *et al.* 2018). Through using this characteristic, seedless mandarins (Cuenca *et al.* 2010), melons (Ezura *et al.* 1993) and grapes (Wakana *et al.* 2003) have been obtained and put into cultivation. The seedlessness requirement of hop became important when almost exclusively hop pellets and extracts started to be used to brew beer instead of pressed cones. The process preceding the production of these hop products requires the cones to be ground thoroughly, which leads to the release of large quantities of fats and proteins from the seeds. These compounds adversely affect the fermentation process of the wort and deteriorate the taste of the beer.

The aim of the present study was to obtain F_1 hybrids from controlled and natural pollination of tetraploid 'Sybilla' forms with diploid pollen, and to assess the cytological status of the obtained plants. An attempt was made to identify which functional characteristics (evidence of suitability for cultivation and processing) are determined by the degree of ploidy. The number of seeds in triploid cones in relation to the diploid cultivar 'Sybilla' was also determined.

Materials and Methods

Research material

Four tetraploids (Syb-tetra 2/8, 2/12, 3/8, and 3/10) obtained from 'Sybilla' one of the most promising aromatic hop cultivars were used as maternal forms (Trojak-Goluch and Skomra 2013). Paternal plants were diploid male *H. lupulus* D8 and D11, which were cultivated in a male nursery and characterized by very good morphological

traits as well as large number of inflorescences. Male plants (NN) which were growing near the breeding plantation also became pollen donors.

Controlled and natural pollination of tetraploid forms

Controlled pollination of female inflorescences of Syb-tetra 2/8 and 3/10 forms with pollen of selected D8 and D11 individuals was carried out. For this purpose, part of inflorescences were protected with paper insulators and then fresh pollen was sprayed inside. The remaining inflorescence of Syb-tetra 2/8, 3/10, 2/12 and 3/8 were subjected to natural pollination by pollen of male individuals (NN). Seeds were harvested from the infructescences at maturity and vernalized in the dark for 3 weeks at 4°C at 80% air humidity. After this period, the seeds were placed in a peat substrate to raise seedlings. When the seedlings reached about 15 cm in length the sex of the plants was assessed.

Molecular identification of the sex of the F_1 hybrid populations

Sex detection of F_1 hybrids was performed using the PCR method. Genomic DNA was isolated using the CTAB method (Doyle and Doyle 1987). The PCR reaction mixture was prepared in 25 μ l and contained 5 μ l of plant DNA, 2.5 μ l PCR buffer, 2.5 μ l $MgCl_2$ (2.5 mM), 1.6 μ l dNTP (0.25 mM), 1.25 μ l each of the 0.5 μ M primers and 0.2 μ l of Taq polymerase (Fermentas). This study used a pair of STS K-22 primers (5'-CAGTGTTTCTCTCGGGTTCTCTTG-3' and 5'-AACCACACATAATTCCCATCTTGC-3') previously developed by Danilova and Karlov (2006). These primers amplified the male specific sequence of 387-bp. The PCR reaction consisted of 30 cycles: 5 min at 94°C, 1 min 30 s at 60°C and 45 s at 72°C with final elongation of 7 min at 72°C. The amplification was preceded by a denaturation at 94°C for 1 min. The PCR product was analyzed on 2% agarose gel with 0.2 μ g/ml ethidium bromide in 1 \times TBE (100 mM Tris, 90 mM H_3BO_3 , 1 mM EDTA, pH 8.5).

Assessment of the ploidy level of the F_1 hybrid populations

The degree of ploidy of the F_1 hybrids was evaluated with a flow cytometer. The diploid 'Sybilla' was used as an internal standard. Leaf tissue (1 cm²) from the internal standard and a sample were chopped in a Partec buffer enriched with DAPI (Sigma) at 2 μ g/ml (Śliwińska 2002). The mixture was filtered through a 35 μ m of nylon mesh, incubated at room temperature for 5 min and then the fluorescence of nuclei was collected using a 550-nm Dichroic LP and 465-nm BP of Cell Lab Quanta TM SC cytometer (Beckman Coulter). Each sample was analyzed three times and a minimum of 5000 nuclei per sample was measured.

Cytological analyses of the F_1 hybrid populations

For observation of mitotic chromosomes, leaves of about 0.5 cm² were treated with 0.44% 8-hydroxyquinoline with the addition of 100% maltose in 6:1 (v/v) at 22°C for 5 h. Then the material was fixed in ethanol-chloroform-acetic

acid 6:3:1 (v/v), for 24 h at 22°C and hydrolyzed in 6:1:3:2 (v/v) ethanol-chloroform-acetic acid-hydrochloric acid at 60°C for 5 min. The microscopic preparations were made using the Burns method (1964). Chromosome number was counted from 10 cells at metaphase from 35 plants of each hybrid combination and captured on a digital camera (Nikon DS-5Mc).

Morphological characteristics of triploids

Triploid female individuals were field planted in 2015. After reaching their full fruiting in 2017–2018, biometric measurements were carried out. The distance between the ground and the place where the first fructiferous branches were formed (3 measurements for each plant), the length of fructiferous branches and the length of internodes (6 measurements for each plant), the shoot twist index, i.e. the number of shoot turns on a 1 m long section located at the height of 1 to 2 m from the soil surface (3 measurements for each plant), were recorded. Additionally, the length of cones and the number of seeds in 60 cones per plant were determined, as well as the number of days from the cutting of the rootstock to the technological maturity of cones.

Analysis of alpha acid content in triploid cones

Cones at the stage of technological maturity were collected from a height of 4–5 m, dried at a temperature of 50°C for 72 h and then ground. Hop dust (10 g ± 0.001) was extracted in 10:2:4 (v/v) toluene–methanol–HCl (0.1 M) for 40 min. The assessment of alpha acid content (mg/g dry weight) was performed by HPLC using an Agilent Technologies 1200 apparatus. The chromatographic separation was conducted on EC 125/4 Nucleodur RP C18 column (5 µm × 250 × 4 mm). The determination of alpha acid content was performed with a UV/VIS detector at a wavelength of 314 nm. The injection volume was 5 µl and flow velocity of the mobile phase was maintained at 1 ml/min. Each sample was analyzed in triplicate. The identification and calculation of alpha acid concentration was carried out by comparing the retention times of the sample using International Calibration Extract (ICE3, Labor Veritas, Switzerland) of known composition of bitter acids.

Statistical analysis

The results were analyzed using one-way ANOVA variance analysis, in which the year × hybrid combination interaction was taken into account. The significance of differences between the triploids was analyzed using the Fisher post-hoc test at the significance level $p = 0.05$. The Statistica 8.0 (StatSoft) program was used.

Results

Controlled pollination of Syb-tetra 2/8 and 3/10 plants with D8 and D11 pollen as well as open pollination of Syb-tetra 2/8, 2/12, 3/8 and 3/10 forms resulted in fully developed seeds. The number of viable plants ranged from minimum of 53 for Syb-tetra 3/8 × NN to maximum of 67 for Syb-tetra 2/12 × NN hybrids (Table 1). Cytometric analyses of six F₁ hybrid populations revealed the presence of three polyploid groups: diploids, triploids and tetraploids (Supplemental Fig. 1). Cytological evaluation showed, however, that in the group of individuals previously classified in the cytometric method as triploids there were plants containing three times the basic number of chromosomes ($2n = 3x = 30$), as well as aneuploids (Table 2). The frequency of aneuploids with the genetic formula $2n = 3x + 1 = 31$ was 3.8%, while the individuals with $2n = 3x - 1 = 29$ or $2n = 3x - 2 = 28$ chromosomes (Fig. 1) constituted 8.6 and 1.9%, respectively. In the groups classified as diploids and tetraploids, 20 and 40 chromosomes were found respectively, which confirmed the cytological status of the plants.

PCR reaction using STS primers (K-22) coupled with the male specific fragment showed a predominance of female plants among the F₁ hybrids (Fig. 2). In the Syb-tetra 3/10 × D8 combination, the ratio of female to male plants was 1.03:1, while in the offspring of the Syb-tetra 3/10 × NN plants it was 3.71:1 (Table 1). At a significance level of 0.05, the value of χ^2 statistics as well as the value of P showed that the observed ratio of female to male plants differed significantly from the ratio 1:1 characteristic for the majority of dioecious species.

Taking into account the cytological status of plants and their sex, 74 triploid female individuals were selected.

Table 1. Ploidy level and frequency of female plants in F₁ hybrids of tetraploid and diploid forms of *H. lupulus* L. ‘Sybilla’

Hybrid combination	Number of F ₁ hybrids	Ploidy (%)			Female plants (%)	♀:♂	χ^2 *
		Diploid	Triploid	Tetraploid			
Syb-tetra 2/8 × D11	62	0	98.4	1.6	72.5	2.65:1	12.64
Syb-tetra 3/10 × D8	59	1.7	98.3	0	50.8	1.03:1	0.02
Syb-tetra 2/8 × NN	63	0	100	0	57.1	1.33:1	1.29
Syb-tetra 2/12 × NN	67	0	100	0	77.6	3.46:1	20.43
Syb-tetra 3/8 × NN	53	1.9	98.1	0	64.2	1.79:1	4.25
Syb-tetra 3/10 × NN	66	1.5	97.0	1.5	78.8	3.71:1	21.88
Total	370	0.9	98.6	0.5	67.3	2.05:1	44.28

* Significance level $p = 0.05$; D8, D11, NN: diploid male individuals.

Table 2. Cytological analysis of F₁ hybrids of tetraploid and diploid forms of *H. lupulus* L. ‘Sybilla’

Hybrid combination	No. of F ₁ hybrids	Number of plants with chromosome number:						
		20	28	29	30	31	32	40
Syb-tetra2/8 × D11	35	0	1	2	30	1	0	1
Syb-tetra3/10 × D8	35	1	1	2	29	2	1	0
Syb-tetra2/8 × NN	35	0	1	1	29	3	1	0
Syb-tetra2/12 × NN	35	0	1	4	29	1	0	0
Syb-tetra 3/8 × NN	35	0	0	4	31	0	0	0
Syb-tetra 3/10 × NN	35	0	0	5	28	2	0	0
Total	210	1	4	18	176	8	2	1
(%)	100	0.5	1.9	8.6	83.8	3.8	0.9	0.5

D8, D11, NN: diploid male individuals.

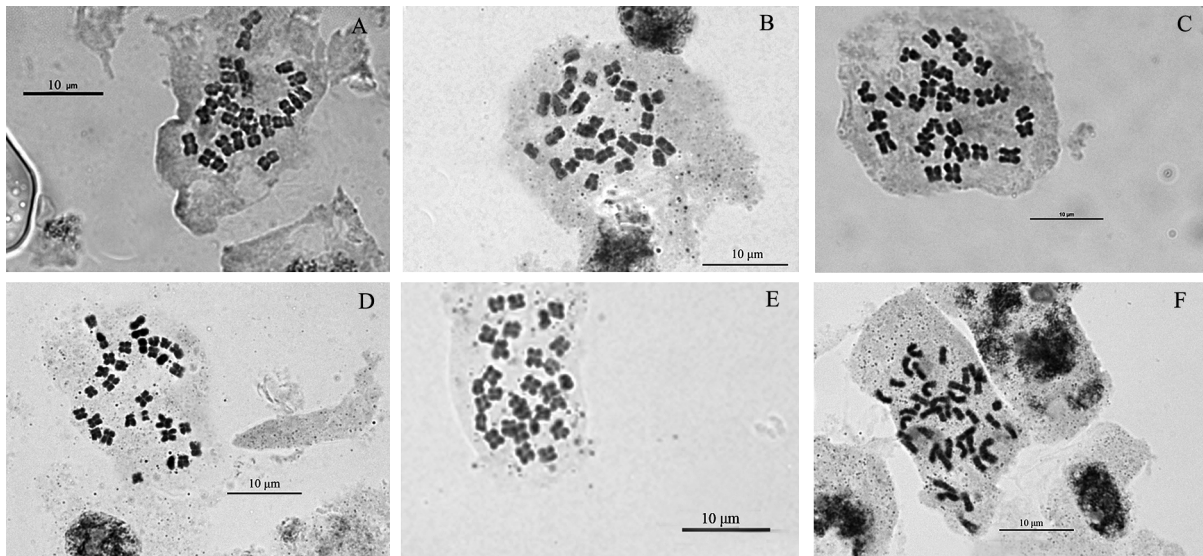


Fig. 1. Mitotic metaphase chromosomes of F₁ hybrids of tetraploid and diploid forms of *H. lupulus* ‘Sybilla’: (A) 28 chromosomes ($2n = 3x - 2 = 28$), (B) 29 chromosomes ($2n = 3x - 1 = 29$), (C) 30 chromosomes ($2n = 3x = 30$), (D) 31 chromosomes ($2n = 3x + 1 = 31$), (E) 32 chromosomes ($2n = 3x + 2 = 32$), (F) 40 chromosomes ($2n = 4x = 40$), bar = 10 µm.

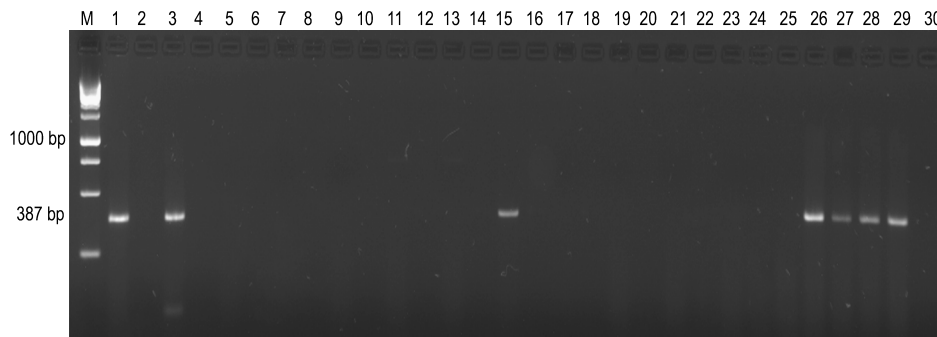


Fig. 2. PCR analysis in F₁ hybrids of tetraploid and diploid forms of *H. lupulus* ‘Sybilla’ by STS K-22 primers linked with male sequence. Lane M 1000-bp molecular marker; lines 1, 3, 15, 26, 27, 28, 29 male specific bands, lines 2, 4–14, 16–25 female individuals, lane 30 negative control (water).

Biometric measurements under field conditions in 2017–2018 showed that fructiferous branches of the triploids obtained from natural pollination were located significantly higher than in ‘Sybilla’ (Table 3). In turn, triploids obtained from the controlled pollination were similar in this respect

to the control cultivar, meaning that they formed fructiferous branches on a longer part of the shoot, starting from near the ground and higher. Hybrid combination × year interaction was significant ($F = 4.05$) for that trait in the case of triploids of Syb-tetra 3/10 × D8 and 2/12 × NN. The length

Table 3. Morphological traits and alpha acids content in F₁ hybrid triploids of tetraploid and diploid forms of *H. lupulus* L. ‘Sybilla’

Hybrid combination	HPFS (cm)	LFS (cm)	Length of internodes (cm)	Shoot twist index	Length of hop cones (cm)	Number of seeds per cone	Number of days to TMC	Alpha acid content (% d.w.)
Syb-tetra 2/8 × D11	79.04 (3.5) ^{ab*}	41.62 (1.3) ^a	16.65 (0.3) ^a	11.68 (0.3) ^{bc}	26.05 (0.4) ^a	2.39 (1.9) ^c	147.6 (0.5) ^b	5.08 (0.2) ^b
Syb-tetra 3/10 × D8	73.00 (6.4) ^a	59.03 (3.1) ^b	16.43 (0.7) ^a	11.36 (0.5) ^{bc}	26.39 (1.2) ^a	3.09 (4.5) ^d	147.5 (1.3) ^b	5.00 (0.3) ^b
Syb-tetra 2/8 × NN	119.67 (5.1) ^d	78.98 (3.1) ^c	23.21 (0.5) ^d	8.05 (0.3) ^a	23.30 (0.4) ^c	1.46 (2.5) ^a	148.4 (0.5) ^{bc}	4.91 (0.4) ^b
Syb-tetra 2/12 × NN	103.63 (5.6) ^{cd}	65.62 (1.9) ^b	21.09 (0.5) ^c	12.06 (0.4) ^c	23.50 (0.3) ^c	1.41 (1.8) ^a	149.9 (0.9) ^{cd}	3.84 (0.3) ^c
Syb-tetra 3/8 × NN	97.24 (5.7) ^{bc}	63.94 (2.6) ^b	23.58 (0.7) ^d	11.24 (0.6) ^{bc}	24.64 (0.7) ^{bc}	1.84 (3.7) ^{ab}	151.7 (0.8) ^d	4.96 (0.2) ^b
Syb-tetra 3/10 × NN	103.32 (7.8) ^c	73.46 (2.6) ^c	18.88 (0.6) ^b	11.29 (0.6) ^{bc}	24.41 (0.6) ^{ab}	2.21 (3.3) ^{bc}	154.6 (1.0) ^e	5.71 (0.6) ^a
‘Sybilla’ diploid	73.78 (5.0) ^{ab}	50.78 (4.5) ^{ab}	14.33 (0.7) ^a	9.33 (0.4) ^a	25.05 (0.6) ^{bc}	17.75 (3.9) ^e	142.0 (1.0) ^a	5.02 (0.2) ^b

* Data represents mean and (SE) values.

^{a-e} Means in columns followed by different letters differ significantly from ‘Sybilla’ based on Fisher’s test.

D8, D11, NN: diploid male individuals, HPFS: height of position of fructiferous branches, LFS: length of fructiferous branches, TMC: technological maturity of cones.

of fructiferous branches is also a parameter determining the usefulness of hop for cultivation. Lateral shoots reaching 50–90 cm in length can produce a large number of well-developed cones and enable efficient cutting of plants on the plantation and machine harvesting. Regardless of the pollen donor used, most triploids had longer lateral shoots than ‘Sybilla’, however only in the case of Syb-tetra 2/8 × NN and 3/10 × NN, the differences were statistically significant. A significant interaction between hybrid combination × year for Syb-tetra 2/8 × NN was found, which indicates the sensitivity of the line to changing climatic conditions. An important criterion for assessing the suitability of hybrids for cultivation is the length of the internodes. The shorter the internodes are, the higher the number of fructiferous branches. The length of internodes of triploids obtained under the controlled pollination did not differ from the value obtained for ‘Sybilla’, while individuals originating from free pollination had significantly longer internodes.

Almost all triploids were characterized by a significantly higher shoot twist index than the diploid cultivar. Detailed analysis showed a significant interaction of the hybrid combination × year ($F = 5.55$). All triploids reached technological maturity of their cones significantly later than ‘Sybilla’. In the population of triploids there was variation in cone length (Fig. 3), ranging from 23.30 mm to 26.39 mm (Table 3). Significantly longest cones was observed in plants originating from the controlled pollination of Syb-tetra 3/10 × D8 and 2/8 × D11. Triploids were characterized by a significantly smaller number of seeds in cones compared to ‘Sybilla’. Statistically significant differences in the number of seeds between hybrid combinations were also found. Noteworthy are the hybrids derived from free pollination of Syb-tetra 2/12 × NN and 2/8 × NN, whose number of seed per cone was only 1.41 and 1.46, respectively.

The HPLC method used in the study showed differences in alpha acids content among triploid populations ranging from 3.84 to 5.71% of dry weight (Table 3). Most plants contained a similar amount of alpha acids as ‘Sybilla’ with

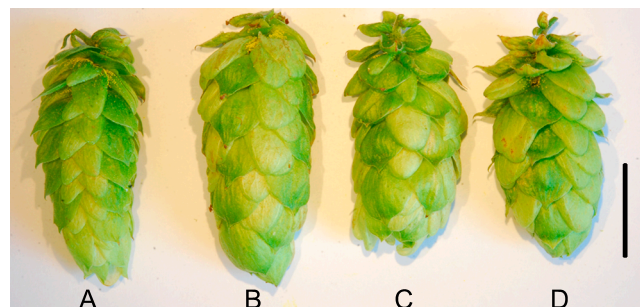


Fig. 3. Hop cones of F₁ hybrids of tetraploid and diploid forms of *H. lupulus* ‘Sybilla’: (A) hop cones of diploid ‘Sybilla’, (B–D) hop cones of triploid plants, bar = 1 cm.

only the triploids of Syb-tetra 2/12 × NN having significantly less of these compounds. The population of Syb-tetra 3/10 × NN plants stood out in terms of their high content of alpha acids.

Discussion

Cytometric and cytological analysis of F₁ hybrids obtained from crossing tetraploids of ‘Sybilla’ with diploid males showed that most of the offspring were triploids containing three times the basic number of chromosomes. However, a small percentage of aneuploids was noted. The presence of plants containing 28, 29, 31 and 32 chromosomes among 4x × 2x hybrids was probably caused by unequal segregation during meiosis I, which resulted in the loss of chromosomes or the presence of additional ones in gametes of tetraploid maternal forms. The presence of 40 chromosomes in the F₁ hybrids indicates that the tetraploid maternal forms can produce euploid gametes of n = 30 (containing 20 plus 10 extra chromosomes), the combination of which with pollen from diploids (n = 10), results in the formation of tetraploids. So far, cytological studies of hops have been undertaken only by Haunold (1972). He included cytological analysis of F₁ hybrids obtained as a result of controlled pollination of tetraploid forms of the

'Fuggle' with diploid pollen. In the $4x \times 2x$ type offspring, triploids (76.3%) containing 30 chromosomes in somatic cells were also dominant while plants distinguished by the absence of one chromosome ($2n = 3x - 1 = 29$) or the presence of an additional chromosome ($2n = 3x + 1 = 31$) constituted 7.5 and 13.3%, respectively. Haunold (1972) also noted a group of individuals showing a high level of aneuploidy, i.e. 32, 33, 39, 41 and 42 chromosomes. No genotypes with such a high level of aneuploidy were found in our study, which was probably due to the death of embryos with highly unbalanced chromosome numbers. The decreased viability of aneuploid has been observed in maize (Cooper and Birchler 2001). It was shown to be a result of trans-acting dosage effects that reduced gene expression. Cytological analysis of $4x \times 2x$ and $2x \times 4x$ hybrids have been conducted for *Lilium pumilum* (Zhang *et al.* 2018). Only triploids containing $2n = 3x = 36$ chromosomes were obtained after crossing diploids ($2n = 2x = 24$) with tetraploids ($2n = 4x = 48$). Similarly Wakana *et al.* (2003) reported that interploid crosses between diploid and tetraploid grapes resulted only in triploids ($2n = 3x = 57$). Aleza *et al.* (2012) showed that in the population of mandarins obtained from $4x \times 2x$ crossings only triploids (98.3%), diploids, tetraploids and pentaploids were found. However, no aneuploids were observed. The relatively high percentage of aneuploids observed in Haunold (1972) and our studies indicates that hop seems to be a species showing much greater tolerance to chromosome imbalance than other species.

Screening carried out on hop seedlings using STS K-22 markers allowed recognizing the sex of a given plant much earlier instead of waiting until plant blooming. It also demonstrated the numerical predominance of female plants in the F₁ hybrids. The ratio of female to male individuals was 2:1 on average. A high frequency of females has previously been observed by Haunold (1972) in $2x \times 2x$ and Beatson *et al.* (2003) in $3x \times 2x$ hybrids of hop. Haunold (1971) and Smith (1963) reported that the predominance of females may be caused by the presence of genes whose expression causes the dying off of pollen containing the Y chromosome. Similar disorders were observed in *Rumex acetosa* and *Silene latifolia* that have heteromorphic chromosomes of the sexes (Charlesworth 2002). The degeneration of Y chromosome led to a weaker germination of pollen and was the reason for the predominance of female plants.

The literature on hop lacks reports on the morphological traits of triploids. Most of manuscripts have focused on yield potential, yield chemistry as well as flavor attributes (Beatson *et al.* 2003, Beatson and Alspach 2007, Probasco *et al.* 2006). In this trial, triploids were characterized by significant differences in their morphological features, which is a result of their hybrid character. Individuals obtained from the controlled pollination produced fructiferous branches over a longer part of the main shoot, while triploids derived from natural pollination formed their fructiferous branches higher. The absence of fructiferous

branches at the bottom of these plants may cause a decrease in their yield. Our studies revealed that despite the high level of variation among triploids, they were able to produce longer fructiferous branches compared to the diploid 'Sybilla'. Vandenhout *et al.* (1995) also reported that banana triploids produced longer suckers at harvest compared to the diploid and the tetraploid forms. The data showed that the change in the ploidy of hop affected the length of internodes. All triploids had longer internodes compared to 'Sybilla', resulting in loosely arranged fructiferous branches. It was also shown that this negative effect can be lessened by the proper selection of the pollen donor. The results are consistent with the data presented by Chang *et al.* (2018), who reported that in mulberry the internode length of triploids was much longer than in diploids and suggested that this parameter could provide an useful tool for differentiation of individuals with different ploidy levels. One of the important parameters characterizing the usefulness of hop for cultivation is the shoot twist index. The studied populations showed a significantly higher number of shoot turns compared to the cultivar 'Sybilla', which indicates they are more suited for climbing up on wires and fix on them better than diploids, and hence they are protected from sinking to the ground in strong winds. Analysis of the data showed that hybrid combination \times year interaction was significant for that trait. This indicates that a large number of shoot twists is a feature that distinguishes triploids, but it is also dependent on climatic conditions. Polyploidisation of the genome had an important effect on the morphological and chemical characteristics of yield. Triploid apple (Podwyszyńska *et al.* 2016) and mulberry (Chang *et al.* 2018) produced significantly larger leaves and fruits than diploids. Similarly, Vandenhout *et al.* (1995) stated that triploid banana produced about 15 g heavier fruits than their diploid counterparts. Our results correspond well with these findings and showed that the hop cones of populations derived from the controlled pollination were on average longer than those from the plants derived from open pollination and from maternal 'Sybilla'. Due to that, the genotypes Syb-tetra 2/8 \times D11 and Syb-tetra 3/10 \times D8 can be valuable breeding material for obtaining commercial cultivars. We also found that triploidy influenced the length of the plant development stages because triploid cones reached technological maturity significantly later than the diploid 'Sybilla'. The introduction of hops cultivars with a late ripening period would facilitate the organization of work on large-area farms where several hop cultivars with varied ripening period are usually grown.

Most of reports indicate that triploids are generally sterile due to pairing problems in meiosis that results in unbalanced and non-viable gametes (Zhang *et al.* 2018). The sterility that accompanies triploidy has been used in the production of completely seedless and commercially significant mandarin (Cuenca *et al.* 2010), banana (Ortiz and Vuylsteke 1995) and melon (Ezura *et al.* 1993). We

observed that hop triploids were not fully sterile and produced a few seeds, but significantly fewer compared to diploid ‘Sybilla’. It is an important trait determining the high usefulness of triploids in brewing (that allows the production of raw material largely devoid of seeds, even when males cannot be eliminated from the surroundings of hop gardens).

The crosses of tetraploid and diploid forms allowed obtaining six F_1 hybrid populations of *H. lupulus*. Most of individuals were triploids, however a high number of aneuploids was also generated. Assessment of morphological and chemical traits revealed that some triploids performed better than diploid ‘Sybilla’. They produced longer fructiferous branches and longer cones as well as they were very well suited for climbing up on wires due to their high shoot twist index. The most important trait of all triploids was an almost complete seedlessness of cones. These genotypes can be an excellent source of raw material for hop pellets producers and the brewing industry.

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Author Contribution Statement

AT-G performing of the experimental part, statistical analysis of data, editing of the text; US performing of the experimental part, editing of the text.

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