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Breeding of triploid common hop cultivars (*Humulus lupulus* L.)

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Abstract. Genome polyploidisation plays a special role in the progress of crop improvement in agriculture. Duplication of the entire genome is associated with significant phenotypic changes in plants, which most often lead to an increase in production at an unchanged level of input. Triploid hop genotypes are distinguished from diploids by their higher yielding potential, increased alpha-acid content and absence of seeds. For this reason, triploid hop cones are an extremely useful raw material for the brewing industry. Studies on the polyploidisation of hop genomes were initiated by Dark in 1948. In the 1950s, American researchers Neve and Farrar made an important contribution to hop triploid breeding. A significant improvement in yield per unit area and in the quality of hop raw material was brought about by the release of aromatic triploid cultivars: Willamette and Columbia to hop farmers by Haunold et al. in 1977. The development of a method for the induction of tetraploid hops using colchicine in *in vitro* cultures has resulted in a number of valuable high alpha as well as aromatic triploid hop cultivars being obtained in New Zealand. As a result of the breeding work carried out in Slovenia in the 1990s, an array of triploid cultivars was obtained, the introduction of which resulted in a significant increase in the cultivation area of aromatic cultivars in this country. Currently, breeding work aimed at obtaining super alpha and aromatic triploid hop cultivars is being carried out in Poland at the Institute of Soil Science and Plant Cultivation – State Research Institute.

Keywords: polyploidisation of genomes, triploid, *Humulus lupulus,* seedlessness

TRIPLOIDS – SIGNIFICANCE AND METHODS OF OBTAINING

The production of polyploid forms is a widely used method in plant breeding. Plant genomes are very tolerant of increased DNA content, despite many adverse consequences in the form of disturbances in meiosis, changes in the segregation of traits, gamete infertility, or reduced fertility (Comai, 2005). About 70% of known species of angiosperms were created in the course of evolution through polyploidisation (Masterson, 1994). These are mainly forms, whose chromosome numbers constitute even multiples of the basic numbers of chromosomes in genomes, the so-called autopolyploids, as well as interspecific hybrids in which the number of chromosomes in parental genomes doubled, i.e. allopolyploids and auto-allopolyploids (Rogalska, 2012). Natural allotetraploids are, for example, upland cotton, wheat, and autoallopoliploids, strawberry or timothy-grass (Rogalska, 2012). They usually produce larger somatic cells compared to diploid plants, which positively influences the thickness of the stems, the size of leaves and flowers. In addition, they are characterized by an increased content of cell fluid and thinner cell walls, which ensures a better structure of fruit and vegetables (Podwyszyńska et al., 2016). There are also such species for which triploidy is the most beneficial degree of multiplication of genetic material from the commercial point of view. Triploids, containing three haploid genomes, arise most often as a result of natural selection or sexual crossing of a diploid with a tetraploid. To achieve this, the technique of *in vitro* plant regeneration from triploid endosperm cells is also used (Sun et al., 2011). There are also reports on obtaining triploids as a result of a fusion of diploid somatic protoplasts with haploid microspore cells (Pental et al., 1988).

It is believed that the most common causes of a spontaneous appearance of triploids in nature are environmental stress factors, such as: excessive exposure to UV-B radiation, low temperature, shortage of water or nutrients (Mason et al., 2011). Long-lasting and intense influence of frost or the strong stress caused by a drought sometimes leads to errors in meiotic divisions and the production of female gametes with a non-reduced number of chromosomes (2n), which, when combined with haploid pollen (n), give triploid progeny. The ability to create this type of spontaneous triploids is well known among *Populus* species, especially

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Populus tremula, *P. alba* or *P. balsamifera* (Dillewijin, 1939; Gurreiro, 1944; Li, 2001). Triploid plants are characterized by their dynamic growth, large and intensively green leaves, as well as the increased hardness of the trunk in comparison to their diploid counterparts. A similar capacity to produce 2n gametes and spontaneous triploids is demonstrated by potatoes (Wasilewicz-Flis, 2011). As a result of the natural interspecific crossing of Amur silver grass and Chinese silver grass, a new triploid species came into being – giant miscanthus. It is considered to be a species which is particularly valuable for the production of biomass. According to Jeżowski et al. (2007), the triploid giant miscanthus (*Miscanthus sinensis giganteus*) reaches 5 to 6 m in height in its third year since being planted and yields 20–30 tons of dry matter per hectare. The spontaneous crossing of the *Tulipa gesneriana* and *T. fosteriana* species, resulted in the creation of a group of triploid Darwin tulips, significant for commercial reasons (Marasek et al., 2006).

Triploids may also be induced as a result of controlled interploidy hybridization 2x × 2x, 2x × 3x or 4x × 2x. As a result of the hybridization of diploid tangerines (2x × 2x) and the subsequent regeneration of embryos *in vitro*, among others, many triploid citrus hybrids were obtained (Aleza et al., 2010). Mandarins obtained in this way produced seedless fruit with thin skin (Cuenca et al., 2010). In turn, triploid forms of edible cassava produced much higher yields than diploids, and the obtained tubers contained more starch (Hoshino et al., 2011). Another widely used breeding method is the crossing of tetraploids with diploid individuals in 4x × 2x, as well as 2x × 4x systems. By using this method, many new cultivars of the triploid sugar beet, as well as the fodder beet were obtained (Dalke, 2004). Cultivating them allowed for the incremental growth of root yield while maintaining the content of sugars at a level characteristic of diploids (Dalke, 2004). Studies on the hybridization of white mulberry in the 4x × 2x system conducted by Yu et al. (2004) and Yang et al. (2000) made it possible to obtain high-yielding triploids, in which the content of protein in the leaves was 4.14% higher than that recorded in diploid forms. The protein-rich triploid leaves have found their application in silkworm feeding.

In the case of some cultivated species, tetraploid forms (4x) do not exist in nature or are very rare. They may be obtained through the activity of mutagenic agents on somatic tissues both in *in vivo,* as well as in *in vitro* conditions, as a result of the organogenesis in *in vitro* cultures or the fusion of protoplasts obtained through the use of electric current or PEG (Liu et al., 2002; Guo et al., 2006). The most effective and widely used chemical compound which induces polyploidisation is colchicine. It effects by blocking the functioning of the karyokinetic spindle, as a result of which, the chromosomes do not propagate to the daughter cells. The colchicine concentration optimal for polyploidisation varies depending on the species and ranges from 0.25 mM to 38 mM. In addition, the exposure time and the type of tissue treated also influence the effectiveness of this antimitotic agent. However, due to the strong toxicity of colchicine, the use of oryzalin or trifluralin, i.e. herbicides from the group of aniline derivatives, in many research projects is becoming increasingly widespread. A rarely used technique of doubling the genetic material is the process of intermediate organogenesis in *in vitro* conditions or protoplast fusion. It is commonly believed that organogenesis promotes the instability of callus tissue and the occurrence of somaclonal variation in regenerants. Meanwhile, the use of protoplast isolation and fusion technologies to obtain polyploids is difficult from a technical standpoint and inefficient (Eeckhaut et al., 2013).

The regeneration of plants from the parenchymatous tissue of the endosperm is a frequently used technique of triploid production. In most plants, the endosperm created as a result of double fertilization of the central cell is triploid and demonstrates significant totipotency. Work on the breeding of endosperm in *in vitro* conditions was begun by Lampe and Mills (1936). Their studies led to the proliferation of maize endosperm cells. The following years abounded with studies on endosperm cultures as a potential source of triploid plants. In 1965, Johri and Bhojwani (1965) obtained sterile cultures of the *Exocarpos cupressiformis* endosperm, while Johri and Nag (1968) effected the regeneration of shoots from the endosperm of *Dendrophthoe falcata*. Nakano et al. (1975) developed a technique of regenerating the callus, shoots and roots from the endosperm of rice. It was not until a few years later, that the regeneration of a plant representing this species was effected by Bajaj and his team (Bajaj et al., 1980). He showed that immature endosperm cultures are an excellent source of material for triploid regeneration, as a result of both indirect and direct organogenesis. In the following years, intensive research was carried out on *in vitro* cultures of immature endosperm. As a result, triploids of *Morus alba* (Thomas et al., 2000), *Eucalyptus* (Li et al., 2008) and *Phlox drummondii* (Tiku et al., 2014) were obtained, among others. In addition, it was found that the best possible condition for the induction of callus are created by the endosperm collected in the globular or torpedo stage of the embryo (Thomas, Chaturvedi, 2008) and kept on ½ Murashige and Skoog medium enriched with auxins such as: 2,4-D, NAA (1-naphthaleneacetic acid) or IAA (indoleacetic acid), as well as BAP (benzylaminopurine) and KT (kinetin) cytokinins. Meanwhile, the regeneration of shoots and plants benefits from the addition of IAA or NAA, as well as TDZ (thidiazuron) and BA (benzyladeninopurine) to the growing medium (Wang et al., 2016). The authors emphasize, however, that the high proportion of albino and aneuploid plants is a fairly frequent and unfavourable phenomenon associated with the regeneration of plants from endosperm cultures. For example – out of the 80 plants obtained from the *Phlox drummondii* endosperm, 70% were triploids (2n = 3x = 21), 5% diploid (2n = 2x = 14) while the remaining 25% were aneuploid.

DESCRIPTION OF HOP TRIPLOIDS

Common hop *Humulus lupulus* L. included in the order of Urticales, the family of Cannabaceae is a dioecious perennial, whose infructescences are used in the brewing industry for beer production. On the bracteoles of the infructescences (cones), there are lupulin glands in which, among others, soft hop resins accumulate, including alpha- and beta-acids which give the beer its characteristic bitterness and stabilizing foam. Hop cones are also a source of essential oils such as myrcene, humulene, caryophyllene, farnesene responsible for the noble aroma of beer (Agacka, Skomra, 2012). In addition, hops contain secondary metabolites with lower or higher bioactive activity. The most well-known ones are polyphenols, among them xanthohumol, its isomer – isoxanthohumol and desmethylxanthohumol, with strong antioxidant and anticancer effects (Stevens, Page, 2004).

On hop plantations, diploid cultivars predominate (2n = 2x = 20), but in some countries triploid forms are known and cultivated (2n = 3x = 30). In most cases, triploids were created as a result of the controlled crossing of the tetraploid female form of a recognized cultivar with a diploid male (Beatson, Alspach, 2007). A small number of plants with the genetic formula of 2n = 3x = 30 were identified also in the offspring obtained from free pollination of triploids with pollen of diploid plants (Beatson et al., 2003). The obtained plants showed a strong resemblance to the cultivated forms due to the dominance of the genetic material of the mother cultivar in relation to the genome of the paternal form. In hop breeding, this resemblance of triploids to mother specimen is crucial, as it allows for the majority of the beneficial features of the descendant cultivar to be maintained. However, on the other hand, the genome introduced by male plants facilitates, to a greater or lesser extent, the attainment of genotypes with enhanced commercial traits. Hop triploids are distinguished by their intense growth and significant lushness in comparison to diploids. Haunold (1972) studied the increases in the diploid and triploid form of the Fuggle cultivar. He showed that the daily growth of plants with an increased ploidy was 18 mm higher than that of their diploid equivalents. In turn, Krajl (1973) reports that in the cones of the triploid plants from the Atlas cultivar a significantly higher number of lupulin glands ($55.04\pm1.70/cm^2$) is observed than in the diploid mother form ($43.50\pm0.69/cm^2$). Furthermore, the diameter of lupulin glands is much larger, which has a significant influence on the content of soft resins in triploid cones. Prabasco et al. (2006) report that the content of alpha acids in the triploid hop cones of the Millenium cultivar reaches 15.5%, while in the mother diploid Nugget cultivar stands at 13.0%. They also indicate the higher content of humulene and caryophyllene in the essential oils, which imparts a pleasant, hop-like aroma to the beer.

From the perspective of hop producers, one of the most important advantages of triploids is the higher yielding potential in comparison to diploid forms. The data presented in Table 1 shows that the triploid Pacific Jade or Nelson Sauvin cultivars yield twice as high as the mother cultivars (First Choice and Smoothcone respectively). Among the advantages of triploid hop, the fast closure of cones after forming, which limits their exposure to fungal pathogens such as *Pseudoperonospora humuli*, *Podosphaera macularis*, and thus contributes to reducing the number of preventive treatments on the plantation, should also be mentioned. The lower brittleness and fragility of the cones, is another asset of triploids, which allows for the preservation of their quality during mechanical harvesting and transport.

The most important practical advantage of the triploid hop cultivars is their almost complete seedlessness. According to Haunold (1972), in free pollination, the average number of seeds in the triploid forms of the Fuggle cultivar amounted to 1.7, while the diploid form totalled 12.3. It was also found that the average weight of triploid seeds was substantially lower than the weight of diploid seeds (Haunold, Nickerson, 1979). Most of the triploid seeds had no embryo, due to disruptions in the microsporogenesis process, most often manifested by the occurrence of 4 trivalents, 6 bivalents and 6 univalents or 2 trivalentes, 8 bivalents and 8 univalents, and sometimes 10 bivalents and 10 univalents in metaphase I (Haunold, 1970, 1974). The uneven segregation of chromosomes in anaphase I is also mentioned among the causes for the sterility of plants, as well as the presence of one, two, sometimes five lagging chromosomes, which leads to the formation of gametes with an unbalanced number of chromosomes n = 11, 12, 13, ..., 20, ... 30, whose combination results in the low vitality of embryos. When analysing the course of microsporogenesis in *Medicago sativa* triploids (2n = 3x = 48), Binek and Bingham (1970) found from 2.8 to 3.6 trivalents, from 5.0 to 5.9 bivalents and from 2.5 to 4.5 univalents. In meiotic anaphase and telophase I, they observed lagging chromosomes and a high frequency of tetrads containing from two to four micronuclei, as a result of which the plants examined by them were characterised by low pollen vitality. In turn, Schertz and Stephens (1965) compared the course of microsporogenesis in diploid and triploid (2n = 3x = 30) forms of *Sorghum vulgare*. The authors showed a relationship between the disruptions in meiotic divisions and the low vitality of triploid's pollen.

A lot of attention has been paid to the issue of the sterility of hop triploids. Haunold (1974), as well as Haunold and Nickerson (1979) conducted, among others, studies on using triploid pollen to improve the yield and quality of hop raw material on plantations. They proved that the pollination of diploid Brewer's Gold plants with the sterile triploid pollen caused a significant increase in yield per unit of area as a result of increasing the size and weight of hop cones. In the analysed raw material, they also found a significantly lower number of seeds which contained 23% less fat and 14% fewer proteins than in the case of the raw material obtained from free pollination of the Brewer's Gold cultivar with diploid pollen. The infertility of hop triploids and the seedlessness associated with it became increasingly important at a time when changes in beer production technology occurred. When instead of pressed hops, processed hop goods in the form of pellets and extracts started to be used for beer brewing, the presence of seeds in cones became particularly undesirable. Grinding hop cones along with their seeds resulted in the release of significant amounts of fats and proteins adversely affecting the process of beer fermentation. In good quality raw material, the seed weight should not exceed 2% of the total weight of cones. Otherwise, additional cleaning is required, which significantly increases the cost of producing the pellets. Obtaining seedless hop raw material has become one of the priorities of breeding programs.

THE HISTORY OF BREEDING TRIPLOID HOP CULTIVARS

The first attempts to grow seedless hop were made in England in the 1950s (Dark, 1953). As a result of the effect of colchicine on young hop seedlings, mixoploid plants were obtained. The works were continued by subjecting the obtained plants to free pollination, which resulted in obtaining a small population of triploids. In the 1960s, American researchers Neve and Farrar (1954) made significant contributions to the breeding of hop triploids. In turn, by crossing tetraploid forms of the Fuggle cultivar with diploid males, Haunold (1971) obtained 778 hybrid plants, the majority of which (76.3%) were triploids ($2n = 3x = 30$) and 20.8% were aneuploids ($2n = 3x\text{-}1 = 29$, $2n = 3x+1 = 31$). He sporadically found plants containing 28, 32, 33, 39, 41 and 42 chromosomes. According to the author, the presence of plants with a highly unbalanced number of mitotic chromosomes was a result of the fact that mother forms produced a large number of gametes containing additional chromosomes, as well as gametes, whose number of chromosomes was not reduced. In the examined hybrid population, he observed the prevalence of female plants, which constituted 68.5% of all the examined plants, while the share of male plants accounted only for 2.6%. Among the tested hybrid forms, there were also monoecious plants (28.9%). A similar distribution of sexes with the prevalence of females and a small share of male hop plants was observed by Neve (1961) in tetraploid × diploid hybrids. Haunold's experiments led to the attainment and introduction into cultivation of two aromatic triploid cultivars of hops in 1977: Willamette and Columbia, characterized by their intense growth and high yield (Haunold et al., 1977). The subsequent years of work at the Oregon State University allowed Haunold to obtain, on the basis of the aromatic Hallertauer Mittlefrüh cultivar, triploid hop cultivars, of which Crystal, Mount Hood and Liberty yield almost twice as high as the cultivar from which they were derived (Haunold et al., 1995). When analysing the chemical composition of cones, it can also be seen that the triploid cultivars mentioned, are characterized by a higher content of alpha- and beta-acids than Hallertauer Mittlefrüh (Table 1). They are also characterized by a higher content of hop oils. Since 1998, Henning from the Oregon State University has been involved in the work on hop polyploids, who obtained the cultivar Triplepearl and introduced it into cultivation in 2013 (https://www.ars.usda.gov/pacific-west-area/corvallis-or/forage-seed-and-cereal-research/people/john-henning/). It is a high alpha-acid form obtained on the basis of a tetraploid of the Perle cultivar. It exceeds its mother form in terms of the content of alpha-acids and hop oils. It is also distinguished by a mild and pleasant aroma reminiscent of oranges mixed with lime, honeydew, a hint of melon, resin and pepper.

Big achievements in the breeding of triploid hop cultivars have also been noted in New Zealand. The local breweries demanded seedless cones because the beer "hopped" with seedy raw material was too dark and heavy. Meanwhile, it was the light beers which were popular among consumers. Hence, in 1954, Roborgh began experiments to obtain tetraploid forms of the Cluster and California Late cultivars *in vivo* by using colchicine. Works on polyploids were continued by Frost, who, as a result of free pollination of the Smoothcone tetraploids, obtained and then in 1972 introduced into cultivation the triploid Green Bullet cultivar high in alpha acids (https://learn.kegerator.com/green-bullet-hops/). Another person also involved in the production of triploid hops in New Zealand in the 90s. was Beatson, who obtained a number of valuable cultivars, including the high-alpha Pacific Gem and the aromatic Wakatu and Pacifica (Beatson, Brewer, 1994). As has been shown by research, the obtained cultivars were almost seedless and contained much larger amounts of alpha acids than their parental forms (Table 1). In the years 2004–2011 at the Horticultural Research Center, many triploid cultivars (Pacific Jade, Rahau, Kohatu and Wai-iti and others) were obtained, which were characterized by their high content of hop oils (Table 1) and were very popular among hop producers. Among the latest New Zealand cultivation achievements the highly-bitter Waimea obtained in 2012 (16–19% alpha acids) and the Brooklyn cultivar in 2015 ought to be mentioned. The latter differs from its mother form Southern Cross primarily in terms of the high content of alpha acids (17.5–19.5%) and beta acids (8–10%). It is also characterized by its excellent aroma reminiscent of grapefruit, tropical fruit and passion fruit (https://www.yakimachief.com/wp-content/uploads/Yakima-Chief-Hops-Varieties.pdf).

Table 1. Yields and content of soft resins, essential oils in the selected triploid hop cultivars and their diploid maternal forms.

Genetic formula	Cultivar	Yield [t ha^{-1}]	Alpha-acids [%]	Beta-acids [%]	Cohumulone [%]#	Essential oils [ml [100 g]$^{-1}$]	Humulene [%]	Caryophyllene [%]	Myrcene [%]
2n = 2x = 20	H. Mittlefrüh	0.8–1.1	3–5	4–5	20–21	0.7–0.8	30–35	9.9	40.2
2n = 3x =30	Wakatu	1.8–2.0	6.5–8.5	8.4–8.6	28–30	0.9 –1.1	16.7–16.9	8.2	35.5
	Wai–iti	–	2.5–3.5	4.5–5.5	22–24	1.5–1.7	27.9–28.1	9.0	3.0
	Pacifica	1.7–1.8	5–6	5.9–6.1	24–26	0.9–1.1	50.8–51.0	16.7	13.0
	Kohatu	–	6–7	4–5	22–21	0.9–1.0	35.0–36.5	11.5	35.5
	Liberty	1.1–1.7	4–6	3–4	24–28	1.2–1.3	35–40	11.3	46.0
	Mt.Hood	1.7–2.0	5–7	6–7.5	21–23	1.2–1.7	26–36	14.0	35.0
	Crystal	1.3–1.7	3.5–5.5	5.8–7.5	21–26	1.0–1.5	20–26	8.5	47.0
2n = 2x = 20	First Choice	0.9–1.5	4.8–6.7	3.5–6.7	36–39	0.5–1.2	20.7	6.1	55.0
2n = 3x = 30	Pacific Jade	1.8–2.1	12–14	7–8	23–24	1.1–1.4	30–32.9	10.2	33.3
2n = 2x = 20	Smoothcone	0.7–1.5	7–9.5	3.4–5.2	29–31	0.6–1.4	19–20.7	6.1	55.2
2n = 3x = 30	Nelson Sauvin	1.8–2.0	12–13	6–8	22–24	0.9–1.1	32–36.4	10.7	22.2
2n = 2x = 20	Atlas	1.1–1.8	9–11	3.2–4.1	36–38	1.3–1.6	9–11	4.0	49.0
2n = 3x = 30	Blisk	1.5–2.3	9.7–14.1	3.3–4.8	30–33	1.4–2.0	11.5–12	3.7	58.0
2n = 2x = 20	Savinja Golding	1.2–1.8	2.8–5.5	2–3	25–30	0.3–1.0	34–38	9–11	27–33
2n = 3x = 30	Celeia	1.7–2.0	3–6	2–3.3	26–29	1.3–3.6	18–23	8–9	35–40
2n = 2x = 20	Perle	1.5–1.9	7.0–9.5	4.0–5.0	–	0.7–0.9	28–34	12–16	25–35
2n = 3x = 30	Galaxy	–	13.5–14.8	5.8–6.5	–	2.2–2.4	1–2	7–9	33–42

Source: Hop Varietal Guide 2013, Yakima Chief, Inc. (https://www.yakimachief.com/wp-content/uploads/Yakima_Chief_Hops_Varieties.pdf (accessed 05.07.2018)
percentage of alpha-acids

Table 2. The percentage of triploids in the hop growing area (2014).

Country	Area of hop cultivation [ha]	Area of triploid cultivation [ha]	The percentage of triploids cultivation [%]	Number of cultivated triploid cultivars
New Zealand	370	237	64.1	17
Slovenia	1216	441	36.3	7
Australia	408	143	35.0	4
USA	15 564	715.2	4.6	10

Source: the report of the Economic Commission IHGC, 2014 (http://www.hmelj-giz.si/ihgc/doc/IHGC%20hop%20supply.pdf)

The next country with significant achievements in breeding and cultivating triploid hop cultivars is Australia. Works in this area began there at the Tasmanian University in the 1980s. The first polyploid cultivar, Topaz, was obtained in 1985, and the next, Super Pride, in 1987 (http://www.hopslist.com/hops/aroma-hops/topaz/). They were introduced into cultivation by Simon Whittock. This breeder also contributed to the triploid cultivars Ella and Galaxy (Whittock, Koutoulis, 2010). The latter significantly exceeds the original cultivar of Perle in terms of the content of alpha- and beta-acids (Table 1). However, its biggest advantage is the high content of hop oils and a unique aroma that is a combination of citruses with a hint of passion fruit and blackcurrant.

An important centre involved in the cultivation of triploid hop cultivars is the Institute of Hop Research and Brewing in Žalec, Slovenia, where, in 1980, the Blisk cultivar was obtained (Neve, 1986). The breeding of triploids in Slovenia blossomed in the 1990s thanks to Krajl, who obtained and popularized a group of aromatic cultivars, such as: Cekin, Celeia, Cerea, Cicero (Patzak, Henychová, 2016). However, Krajl's experimental work did not find a continuator.

In Poland, breeding work aimed at obtaining triploids of hops were started at the end of the 1950s at the Higher School of Agriculture in Lublin (Tarkowski, Zub, 1959). Subsequent research was conducted in the 1990s, but unfortunately, it did not result in the submission of triploid cultivars to COBORU (the Polish Research Centre for Cultivars Testing). Currently, cultivating works on obtaining bitter and aromatic triploid cultivars of hops are being carried out at the Institute of Soil Science and Plant Cultivation – State Research Institute in Puławy.

Breeding work carried out at the research centers in Australia, New Zealand, the United States and Slovenia led to the development and release of several dozen triploid hop cultivars. They are very popular among hop producers. Currently, they represent over 64% of the hop growth area in New Zealand. Their significant share is also noted in Slovenia and Australia, 35 and 36.3% respectively. On the other hand, the USA have the largest area planted to triploid hops, although its share in the total area under hops constitutes only 4.6% (Table 2).

CONCLUSIONS

The introduction of seedless, triploid hop cultivars into large-scale cultivation seems to be an excellent solution for hop producers and for the brewing industry. The results of the research indicate the high content of resins and hop oils in triploid cones and a reduction in the production costs of hop pellets. In the modern breeding of seedless triploid hop cultivars, *in vitro* cultivation techniques and molecular markers, enabling the rapid selection of desired genotypes and significantly shortening the cultivation process, are used increasingly widely. A very important, but still unsolved problem is the susceptibility of hops to fungal diseases, including powdery mildew. Therefore, it is advisable to cultivate triploids characterized by both a high content of resins and oils and by resistance to diseases.

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