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Vegetative propagation of hops (*Humulus lupulus* L.): Historical approach and perspectives

Propagação vegetativa do lúpulo (*Humulus lupulus* L.): Abordagem histórica e perspectivas

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Highlights

Macropropagation studies mainly address stem cuttings.
Micropropagation studies mainly address the use of plant regulators.
Macropropagation: gaps about the size and origin of cuttings and rooting time.
Micropropagation: a trend of studies related to cryopreservation.

Abstract

Hop (*Humulus lupulus* L.) female inflorescences are important raw materials used to produce beers, cosmetics, and medicines. Vegetative propagation is the preferred way of obtaining seedlings for commercial cultivations as female plants produce more lupulin than male plants, a component of commercial interest. It can be carried out by macropropagation (stem cuttings or rhizomes) or micropropagation. This review aimed to systematize different techniques of hop vegetative propagation, with no time frame, from searches in the main academic research bases: Capes Journal Portal, Scielo, Scopus, Web of Science, Science Direct, Google Scholar, and ResearchGate. Most studies are related to micropropagation, mainly addressing different plant regulators and concentrations, as well as types of explants and culture media, strategies to produce virus-free plants, artificial lighting, and cryopreservation. Experiments with stem cuttings are more common regarding macropropagation, but factors such as size and origin of cuttings, rooting period, and the response of different cultivars need to be better evaluated. Cultivation by cuttings allows the production of clones of female plants and micropropagation the production of virus-free clones in a short time and less physical space. Currently, micropropagation has been widely applied to cryopreservation.

Key words: Cannabaceae. Cuttings. Micropropagation.

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Resumo

As inflorescências femininas do lúpulo (*Humulus lupulus* L.) são matérias-primas importantes utilizadas na produção de cervejas, cosméticos e medicamentos. Como as plantas femininas produzem mais lupulina que as masculinas, componente de interesse comercial, a propagação vegetativa é a forma preferencial de obtenção de mudas para os cultivos comerciais. Esta pode ser realizada por macropropagação (estaquia caulinar ou rizomas) ou micropropagação. O objetivo desta revisão foi sistematizar as diferentes técnicas de propagação vegetativa do lúpulo, sem recorte temporal, a partir de buscas nas principais bases de pesquisa acadêmica: Portal de Periódicos Capes, Scielo, Scopus, Web of Science, Science Direct, Google Acadêmico e Research Gate. A maioria dos trabalhos são relacionados à micropropagação, abordando principalmente diferentes reguladores vegetais e concentrações, além de tipos de explantes e meios de cultura, estratégias para produzir plantas livres de vírus, iluminação artificial e criopreservação. Quanto à macropropagação, experimentos com estaquia caulinar são mais comuns, porém fatores precisam ser melhor avaliados tais como tamanho e origem das estacas, período de enraizamento e resposta de diferentes cultivares. O cultivo por estacas permite a produção de clones de plantas femininas e a micropropagação a produção de clones isentos de vírus, em pouco tempo e em menor espaço físico. Atualmente, a micropropagação tem sido muito aplicada à criopreservação.

Palavras-chave: Cannabaceae. Estaquia. Micropropagação.

Introduction

Hops (*Humulus lupulus* L.) is an herbaceous, perennial, dioecious plant belonging to the family Cannabaceae (Behre, 1998; Almaguer, Schönberger, Gastl Arendt, & Becker, 2014). The female inflorescences, called cones, are important raw materials used mainly in the beer production process, in addition to cosmetics and medicines (Biendl et al., 2014). Hop active substances used in the brewing industry (alpha and beta acids and essential oils) are found in the cones, specifically in the lupulin glands, providing the characteristic bitter taste and aroma of this beverage (Batista, Souza, & Pais, 1996; Taniguchi et al., 2014).

Lupulin is present at higher amounts in unfertilized female flowers (Durello, Silva, & Bogusz, 2019), showing high heterozygosity since the species is dioecious. Thus, although hop propagation can be performed by seeds, rhizomes, cuttings, or micropropagation (Sommer et al., 2019), populations obtained by seeds are not recommended for commercial crops. Male plants are very little productive and plants from seeds are very variable, losing the characteristics related to the commercial value of the cultivar that originated them. Propagation via seeds is used only to originate populations for selection in genetic improvement programs. Thus, *H. lupulus* L. used in commercial crops is propagated vegetatively mainly from herbaceous cuttings and rhizomes or by micropropagation (Denoma, 2000; Fagherazzi, Santos, Santos, Rufato, & Moreira, 2018; Nguyen, Vu, Huo, & Pearson, 2020).

Propagation via cuttings consists of using a segment of the plant with at least

one bud capable of forming a plant identical to the parent plant (Fachinello, Nachtigal, & Kersten, 2009). It is the most used method for vegetative propagation of several herbaceous or woody species. Factors such as the mother plant state, type of cutting, plant regulators, and environmental conditions such as light, temperature, and humidity of the air and soil must be considered for this process to be successful (Hassanein, 2013).

Plant regulators, exogenous chemical substances that can be applied to plants to exert physiological functions of plant hormones, promote the induction of initial meristematic activity, and stimulate the elongation and development of formed roots and shoot formation (Davis, Haissig, & Sankhla, 1988; Hinojosa, 2000). The main regulators used in vegetative propagation by cuttings are auxins and cytokinins. The most commercially used auxins are IBA (indole butyric acid) and NAA (naphthaleneacetic acid).

At the cellular level, auxins have the effects of cell division, elongation, and differentiation, increase in osmotic balance, protein production, and cell permeability, in addition to decreasing cell wall pressure. Cytokinins are capable of inducing the formation and elongation of stalks and stems in callus cultures, production of different adventitious roots in the tissues of leaves and stems freshly removed from the mother plant, and formation of apical dominance (George, Hall, & Klerk, 2008; Alcantara-Cortez, Godoi, Cortés, & Mora, 2019).

Cytokinins are plant regulators involved in the regulation of processes such as cell division, shoot and root development, apical dominance, lateral bud growth, seed germination, and delay in plant organ senescence (Nisler et al., 2010). The most used cytokinins are kinetin, zeatin, and benzyladenine (Alcantara-Cortez et al., 2019).

Hop propagation via herbaceous cuttings is a very practical propagation method, as one herbaceous branch of the plant gives rise to several cuttings. Cuttings should be collected before flowering begins to take advantage of the branches with axillary buds and leaves. The cuttings show root induction after approximately 15 days. After the formation of new shoots, the plants are grown in a greenhouse for 4 to 6 weeks with adequate irrigation and fertilization to allow the field planting (Sposito, Barbosa, Ismael, & Tagliaferro, 2019). Hops can also be propagated through rhizomes, which have a good number of buds, being fractionated, placed in pots to root, and then transplanted (Neve, 1991).

In vitro propagation or micropropagation is also an important method of vegetative propagation in plant species. Its main advantages are the production of large amounts of seedlings or parent plants in reduced time and physical space and with better health (Pasa et al., 2012). However, the protocols that encompass each phase of the micropropagation process for hop cultivation are not yet fully developed, with the total control of variables, such as the use of plant regulators, types of explants, cultivation environment, and nutritional components of the culture media (Santos et al., 2019). The supply of certified seedlings is scarce in the market and producers have resorted to their own production of rhizomes or acquired propagation material in parallel markets, regardless of genetic correspondence and phytosanitary quality (Liberatore et al., 2020a).

This review aims to systematize the different techniques of hop vegetative propagation via macropropagation (stem cuttings or rhizomes) and micropropagation, considering the main academic research databases.

Methodology

Searches were performed in the following databases to carry out the bibliographic review on the state of the art of the vegetative propagation of *H. lupulus* L.: Capes Journal Portal (https://www.periodicos.capes.gov.br), Scielo (https://www.scielo.br/), Scopus (https://www.scopus.com/), Web of Science (https://www.webofscience.com/), Science Direct (https://www.sciencedirect.com/), Google Scholar (https://scholar.google.com), and ResearchGate (https://www.researchgate.net/). The following sets of words were used in all databases: "hop vegetative propagation," "hop cutting," "hop tissue culture," "hop rooting," "hop in vitro multiplication," and "hop organogenesis". In addition, the search was repeated by changing the word hop to the scientific name *Humulus lupulus.* The search was carried out in August and September 2020, with no time frame. All studies that had as a research objective hop vegetative propagation were selected.

Subsequently, the studies were systematized according to the following steps: 1) chronological order to evaluate the evolution of the research over the years; 2) separation of macropropagation and micropropagation articles; 3) classification among macro-and micropropagation articles according to the objectives of the study; 4) macropropagation studies were classified by cutting position, light intensity, temperature, pre-treatment with sugar, leaf area, types of substrates, and plant regulators; and 5) micropropagation studies were classified by the effect of plant regulators, used plant organ, the effect of culture media, evaluation of different cultivars, production of virus-free plants, determination of cryopreservation strategies, somaclonal variation, organogenesis events, and LED lighting.

Thus, the steps described above served as the basis for organizing the topics, tables, and figures of this state-of-the-art article on hop vegetative propagation.

Development

Vegetative propagation methods

The first studies on hop propagation were carried out by Howard (1965) and Howard and Sykes (1966), who worked with cutting rooting aiming to evaluate the size and number of leaves among the main variables for root formation.

Sallie and Jones (1969) first described the micropropagation cultivation technique, with shoot tips to produce virus-free seedlings, introducing the micropropagation method in the research. Years later, Svoboda (1988) described callus formation in the cultivation of upper meristems of hops at high concentrations of auxin NAA and cytokinin benzyladenine (BA) and different compositions of culture media containing IBA and 6-benzylaminopurine (BAP). Micropropagation with hop explants was evaluated by Svoboda (1991).

The number of published studies on culture media, production of virus-free plants, and the first work with cryopreservation increased in the 1990s (Table 1). Callus formation in media containing kinetin, IAA, and gibberellin GA3 was successfully tested by Svoboda (1992a,b). Svoboda (1992c) compared culture media containing IAA, kinetin, and GA3 and IBA, BAP, and GA3. The effects of IBA and zeatin were studied on the morphogenetic reaction of isolated hop meristems by Svoboda (1995). Batista, Sousa and Pais (1996) sought to establish a protocol for plant regeneration from callus comparing different cultivars. B. A. N. Adams, Barbara, Morton and Darby (1996) described the successful elimination of hop latent viroid (HLVd) by meristem culture of plant material stored and cultivated at 2-4 °C for periods of 6 to 21 months. Batista, Ascensão, Sousa and Pais (1999) reported the establishment of a highly productive hop regeneration system based on callus culture in a liquid medium. Martinez, Tamès and Revilla (1999) first reported a dehydration and encapsulation method for in vitro cryopreservation of hop shoots in a medium with alginate and 0.5 M sucrose. Sustar-Vozlic, Javornik and Bohanec (1999) investigated the somaclonal variation that occurs in the process of organogenesis from undifferentiated tissue, and Gurriarán, Revill and Tamés (1999) evaluated the direct and indirect regeneration capacity of two commercial hop varieties.

Table 1
Timeline and number of published studies on the vegetative propagation of *H. Lupulus* L

	Decade					
Study objective	**1960**	**1970**	**1980**	**1990**	**2000**	**2010**
Cutting rooting	2	-	-	-	-	3
Virus-free plants	1	-	-	2	2	-
Regulators	-	-	1	7	5	4
Culture media	-	-	-	7	3	1
Different cultivars	-	-	-	3	5	1
Cryopreservation	-	-	-	1	3	1
Somaclonal variation	-	-	-	1	-	-
Organogenesis	-	-	-	-	1	-
Obtainment of tetraploids	-	-	-	-	1	1
LED lighting	-	-	-	-	-	1

At the end of the last century, following the trend of the 1990s, the number of studies with hop micropropagation continued to increase (Table 1). Fortes and Pais (2000) reported the histological effects that occur in callus formation and internode regeneration. The following year, Roy, Leggett and Koutoulis (2001) and Smylakova, Lipavská, and Patzak (2001) tested different culture media. Cryopreservation was again studied by Reed, Okut, Narver and DeNoma (2003) and Horlemann, Schwekendiek, Höhnle and Weber (2003), establishing a method of regeneration and transformation for the cultivar Tettnanger. Grudzińska and Solarska (2005) and Grudzińska, Solarska, Czubacka, Przybys and Fajbus (2006) carried out preliminary studies on obtaining virus-free plants by the culture of meristems, and Reed (2005) used several genotypes for in vitro storage of virus-free germplasm with cryopreservation. Aynalem, Righetti and Reed (2006) also worked with cryopreservation, seeking to determine a procedure for image analysis of the deterioration of pear shoots applied to hop cultivation. From 2007 to 2009, three works studied the effects of plant regulators: Skof, Bohanec, Kastelec and Luthar (2007), Faltus, Bilavcik, Zamecnik and Svoboda (2007), and Schwekendiek, Hanson and Crain (2009). The studies of the last decade on hop propagation began with Trojak-Goluch, Kawka and Czarnecka (2015), who obtained tetraploid hop strains from calluses. In 2018, studies on cutting rooting were published by Machado et al. (2018), Gomes, Machado, Miola and Deschamps (2018), and Fagherazzi et al. (2018), which were the first studies published by Brazilian researchers on the vegetative propagation of *H. lupulus* L. Yang, Chen and Wang (2019) worked with different cultivars and the effects of plant regulators, selecting five genotypes with semi-hardwood cuttings. Finally, Liberatore et al. (2020b) studied the effects of different regulators on the cultivar Gianni and the obtainment of tetraploids, while Nguyen et al. (2020) evaluated the effect of LED light on increasing leaf area and root length of the cultivar Tettnanger in vitro.

Most of the studies were directed, over the years, to the research of methods of vegetative propagation via micropropagation, with 87.1% of the works published using this technique.

Vegetative propagation by cuttings

Few studies related to the hop vegetative propagation by cuttings were found in the researched databases (Table 2). The pioneering study with hop propagation by cuttings was carried out by Howard (1965) on the rooting of cuttings of the cultivar Eastwell Golding, taken from different positions of the plant and analysis of the effect of different temperatures and light intensity on rooting under greenhouse conditions. The best result relative to the number of roots was obtained with cuttings with two nodes taken from the middle portion of the plant, while the worst result was obtained from cuttings of the most distal region relative to the root. The highest root weight was obtained in treatments without shading and at a temperature of 27 °C.

Table 2
Studies carried out with the propagation of stem cuttings of *H. lupulus* L

Treatment	Number of studies	Reference
Cutting position	1	Howard, 1965
Light intensity	2	Howard, 1965; Howard & Sykes, 1966;
Temperature	1	Howard, 1965
Pretreatment with sugar	1	Howard & Sykes, 1966
Leaf area of cuttings	1	Gomes et al., 2018
Substrates	2	Fagherazzi et al., 2018; Guimarães et al., 2019
Regulators	2	Sommer et al., 2019; Guerreiro & Reis, 2019

Howard and Sykes (1966) published a study using again the cultivar Eastwell Golding to evaluate whether the supply of sugar (2% sucrose, fructose, and glucose solution at the proportion 1:1:2) in the cutting bases would increase the root mass. The highest root production occurred in an environment with high light availability, regardless of having been pretreated with sugar. Overall rooting at low light intensity was lower than at high intensities, but a positive effect of sugar pretreatment was observed, leading to large increases in rooting compared to cuttings without pretreatment.

New studies on hop cuttings were found in the researched databases only in 2018. Gomes et al. (2018) evaluated the rooting of cuttings of the cultivar Chinook, with different leaf areas in environments with and without intermittent mist. Cuttings with a pair of whole leaves, a pair of leaves with half the leaf area, a whole leaf, a leaf with half the leaf area, and without leaves were compared. The highest percentages of rooting in the environment with mist were obtained with cuttings with one (97.5%) or two whole leaves (92.5%) or two leaves with half the leaf area (90%). In contrast, the percentage of rooting was lower in the environment without mist, being higher in cuttings with one (62.5%) or two leaves with half the leaf area (72.5%) and a whole leaf (70%). The treatment without leaves showed a rooting of only 2.5%, regardless of the presence of mist.

Fagherazzi et al. (2018) tested different substrate compositions in the rooting of herbaceous cuttings of the cultivars Columbus and Yakima Gold (pure rice husk, pure peat, and rice husk + peat mixtures at 1:4, 2:3, 1:1, 3:2, and 4:1 proportions). The highest means of the percentage of survival of cuttings were obtained using pure peat or mixtures of peat with rice husk for the cultivar Columbus (82.5 to 100%) and pure peat and all mixtures (62.5 to 100%) except the 2:1 mixture for the cultivar Yakima Gold.

Sommer et al. (2019) tested the efficiency of the biofertilizer Stimulate® (gibberellic acid at 50 mg L^{-1}, indole butyric acid at 50 mg L^{-1}, and kinetin at 90 mg L^{-1}), with concentrations of 0 to 6 mL L^{-1} in herbaceous cuttings from 5 to 10 cm. Significant differences were observed relative to root length (quadratic, with maximum technical efficiency close to 4 ml L^{-1}) and root mass (linear). Guerreiro and Reis (2019) evaluated

propagation with cuttings subjected to different natural sources of plant regulators of sedge extract and seaweed extract (6 and 12 mL, respectively). Cuttings treated with sedge extract (6 mL) showed a 20% survival rate, while those treated with seaweed extract and water (control) showed no rooting.

Guimarães et al. (2019) evaluated seedling propagation of the cultivar Cascade by cuttings in different commercial substrate compositions. The substrate (mixture 1:1) showed a 75% efficiency in the formation of shoots, while mixtures 2:1 of a substrate with humus and substrate with cured bovine manure showed efficiencies of 37.5 and 12.5%, respectively.

Thus, there is a need for more research related to the use of plant regulators for hop vegetative propagation. The best results pointed to the use of stem cuttings, preferably with two nodes, taken from the middle portion of the plant with at least one whole leaf. The cuttings need to be placed in an environment with good availability of light and with mist. There was a need for more research regarding the appropriate type of substrate to be used since few alternatives were evaluated. There is also a lack of information about other methods of rooting cuttings, such as the use of substrates in a floating system or even rooting in hydroponic media. Other gaps were related to the production of seedlings from rhizomes and the lack of information about which cultivars are easier to root by cuttings.

Micropropagation

Hop seedling production using only cuttings presents a certain difficulty to the productive sector due to the dependence on traditional propagation material, which is available seasonally, as rhizomes are dormant in the cold seasons and the shoot is produced at harvest, which occurs near the beginning of autumn (Neve, 1991; Martinez et al., 1999). Thus, micropropagation represents an alternative for obtaining seedlings outside the natural season and in a relatively short time and limited space (Martinez et al., 1999; Liberatore et al., 2020a).

Commercial hop propagation has traditionally been carried out through vegetative techniques, either through rhizomes or herbaceous cuttings. Micropropagation has the advantages of increasing the production rate in less time, reducing the incidence of diseases compared to macropropagation, and allowing the production of plants without seasonal restrictions that limit their growth (Nguyen et al., 2020). Cultivars present different responses in the regeneration media, as demonstrated by A. N. Adams (1975), Robins, Furze and Rhodes (1985), Batista et al. (1996), Gurriarán et al. (1999), and Smýkalová et al. (2001).

Most published research involving micropropagation addresses the study of the use of different plant regulators (especially auxins and cytokinins) applied at different concentrations, either alone or in an association. The second most discussed subject in the studies was the composition of the culture media, followed by the evaluation of the response of different cultivars in vegetative propagation via micropropagation. Other studies were also carried out, involving techniques for the production of virus-free seedlings, cryopreservation of the multiplied material, somaclonal variation, obtainment of tetraploids, lighting effect, and description of the stages of the organogenesis process (Figure 1).

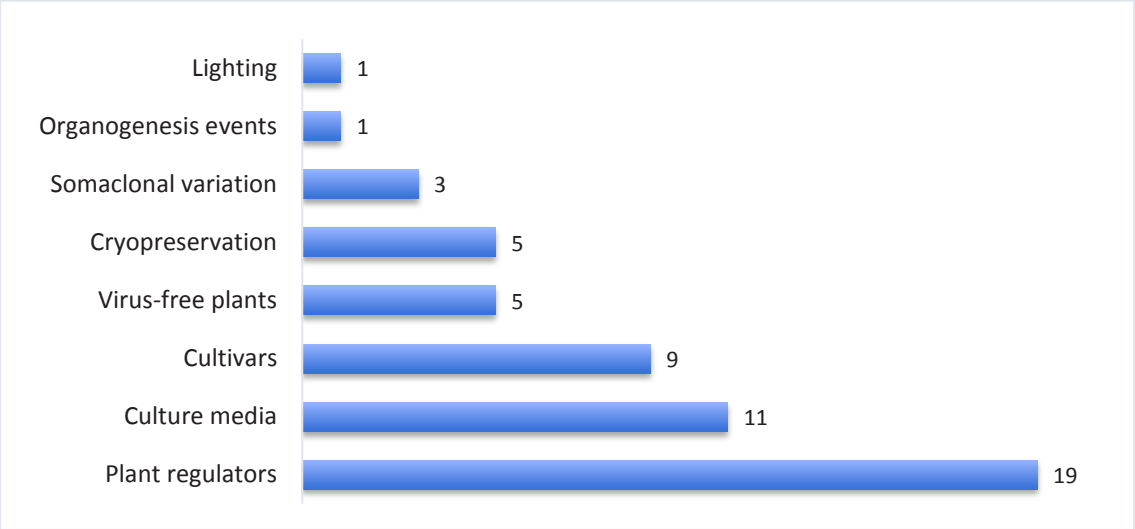

Figure 1. Frequency of objectives of studies related to the micropropagation of *H. lupulus* L.

Effect of plant regulators

The studied plant regulators consisted of the auxins NAA (1-naphthaleneacetic acid), IAA (indole acetic acid), 2,4-D (2,4-dichlorophenoxyacetic acid), and IBA (indole butyric acid) and the cytokinins BA (benzyladenine), 2iP (isopentenyladenine), KIN (kinetin), TDZ (thidiazuron), ZEA (zeatin), ZEAr (zeatin riboside), BAP (6-benzylaminopurine), 6-BA (6-benzylamine), and PUR (purine), in addition to gibberellin GA3. Table 3 summarizes the main results found in the studies. It shows the use of different formulations of culture media, mostly supplemented by different combinations of auxins and cytokinins, in addition to some studies containing isolated applications of cytokinins and the association of auxins and/or cytokinins with gibberellins.

Table 3
Main results obtained in studies evaluating plant regulators in micropropagation of *H. lupulus* L. M (propagation material); PR (plant regulators); C (concentration, 1 µM and 2 mg L^{-1}); SE (sprouting efficiency, %); CF (callus formation, 1 number and 2 %); NS (number of formed shoots); NN (number of formed nodes); R (rooting, %); UC (unidentified cultivar); L (low); N (null); M (medium); H (high); Sp (sprout); Le (leaf); Bu (bud); Ro (root); St (stem); In (internode); Me (meristem); Pe (petiole)

Cultivar	M	PR	C	SE	CF	NS	NN	R	Reference
H138	Sp	NAA+BA	0.54+0.44[1]	79.8	L	1.7	6.3	-	Roy et al., 2001
H138	Sp	NAA+TDZ	0,54+2.27[1]	53.9	M	5.9	25.6	-	
H138	Sp	IAA+BA	0,57+2.22[1]	96.6	N	2.1	11.5	-	
H138	Sp	IAA+2iP	0,57+9.84[1]	85.8	L	1.8	7.8	-	
H138	Sp	IAA+TDZ	0,57+2.27[1]	64.9	L	7.9	41.4	-	

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Tettnanger	Sp	TDZ	4.54[1]	56	1.56[1]	-	-	-	Schwekendiek et al., 2009
Japanese[UC]	Sp	BA+IAA+2,4-D	0.1+1.0+2.0[2]	86.7	-	-	-	-	Yang et al., 2019
Japanese[UC]	Le	BAP+ IAA	0.01+0.1[2]	46.7	-	-	-	-	
Japanese[UC]	Bu	BAP+ IAA	0.01+0.1[2]	51.1	-	-	-	-	
Japanese[UC]	Ro	6-BA + IAA	0.01+0.1[2]	48.9	-		-	-	
200/69	St	KIN+IAA	23.20+0.29[1]	37.5	-	-	-	-	Sustar-Vozlic et al., 1999
Spalter	St	ZEA+IAA	9.12+0.29[1]	31.1	-	-	-	-	
Savinjski	St	ZEAr+IAA	5.69+0.29[1]	26.6	-	-	-	-	
Savinjski	St	KIN+IAA	23.20+1.43[1]	29.2	-	-	-	-	
Savinjski	St	TDZ+IAA	9.08+1.43[1]	20.8	-	-	-	-	
Bragança	St	IAA+ZEA	0.1+3.0[2]	76.9	-	2.9	-	-	Batista et al., 1996
Iunga	St	IAA+2iP	2.85+29.52[1]	90.8	-	19.9	-	-	Trojak-Goluch et al., 2015
Aurora	In	2iP	6.0[2]	17.7	-	2.9	-	-	Skof et al., 2007
Tettnanger	In	2iP	6.0[2]	56.9	-	5.7	-	-	
Tettnanger	In	ZEAr	3.0[2]	30.2	-	21.8	-	-	
Savinjski	In	ZEAr	2.0[2]	40	-	5.2	-	-	
CzechUC	In	ZEA + 2,4-D	1+0.1[2]	-	70.8[2]	-	-	-	Smýkalová et al., 2001
CzechUC	In	KIN+2,4-D	5+10[2]	-	70.3[2]	-	-	-	
CzechUC	In	TDZ	2[2]	-	40.5[2]	-	-	-	
CzechUC	In	PUR+2,4-D	2+0.1[2]	-	40.4[2]	-	-	-	
Brewer Gold	In	ZEA+IBA	4.56+4.99[1]	-	96	-	-	54	Gurriarán et al., 1999
Nugget	In	BAP+IBA	4.4+4.99[1]	-	78	-	-	50	
Tettnanger	In	NAA+BAP	26.85+0.44[1]	-	5.5	-	-	-	Horlemann et al., 2003
Tettnanger	In	IAA+TDZ	0.71+4.54[1]	-	5.7	-	-	-	
Osvald 72	Me	NAA + BA	5-20+1-20[1]	-	H	-	-	-	Svoboda, 1988
Osvald 72	Me	IBA + ZEA	5.0+5.0[1]	-	H	-	-	-	Svoboda, 1995
Clone 72	Me	BAP	1.0[2]	-	M	-	2.2	-	Faltus et al., 2007
Clone 72	Me	BAP+GA_3	1.0+0.2[2]	-	H	-	2	-	
Clone 72	Me	IBA+BAP+GA_3	0.01+0.1+0.02[2]	-	L	-	2.2	-	
Clone 31	Me	IBA+BAP	0.1+1.0[2]	-	M	-	2.8	-	
Clone 31	Me	IBA+BAP+GA_3	0.01+0.1+0.02[2]	-	L		2.8	-	
Iunga	Pe	IAA+BA	0.57+8.87[1]	45.8	-	14.6	-	-	Trojak-Goluch et al., 2015

The best results for sprouting efficiency were obtained by Roy et al. (2001), with a 96.6% sprouting efficiency in a culture medium with auxin (0.57 µM IAA) and cytokinin (2.22 µM BA). A 90.8% efficiency was obtained by Trojak-Goluch et al. (2015) also with an association between auxin (2.85 µM IAA) and cytokinin (29.52 µM 2iP).

Several results with auxins and cytokinins also showed a high organogenic callus formation rate: 4.99 µM IBA + 4.4 µM BAP or 4.56 µM zeatin (78 and 9%, respectively), 5 to 20 µM NAA + 1 to 20 µM BA (Svoboda, 1988), 5 µM IBA + 5 µM zeatin (Svoboda, 1995), and 0.1 mg L^{-1} IBA + 1.0 mg L^{-1} BAP (Faltus et al., 2007). Faltus et al. (2007) also achieved high callus formation rates using the isolated application of cytokinin BAP at a concentration of 1.0 mg L^{-1}, associated with gibberellin (1.0 mg L^{-1} BAP + 0.2 mg L^{-1} GA3), and auxin, cytokinin, and gibberellin (0.1 mg L^{-1} IBA + 1.0 mg L^{-1} BAP + 0.2 mg L^{-1} GA3).

The best results for the number of shoots per explant were obtained by Trojak-Goluch et al. (2015) for the association between auxins and cytokinins (2.85 µM IAA + 29.52 µM 2iP), reaching 21.8 shoots per explant, and Skof et al. (2007), with 21.8 shoots per explant when using 3 mg L^{-1} of the cytokinin zeatin riboside applied alone.

The highest means of the number of formed nodes were obtained by Roy et al. (2001), reaching 41.4 nodes when using the association between IAA and TDZ (0.57 + 2.27 µM, respectively) in a culture medium.

The best results among the studies that compared the percentage of rooting were obtained by Gurriarán et al. (1999) when using associations between auxin and cytokinin at the proportion of 4.99 µM IBA and 4.56 µM zeatin, with 54% rooting for the cultivar Brewers Gold, and 4.99 µM IBA + 4.4 µM BAP, with 50% of rooting for the cultivar Nugget.

The treatments in which the authors tested only cytokinins, probably with higher endogenous auxin content, did not show satisfactory sprouting, with the highest means of only 56.9% (Skof et al., 2007) and 56% (Schwekendiek et al., 2009) for the regulators 2iP (6 mg L^{-1}) and TDZ (4.54 µM), respectively, both for the cultivar Tettnanger (Table 3).

Unlike the studies listed in Table 3, Machado et al. (2018) performed ex vitro application of plant regulator in shoots from micropropagation. Concentrations between 0 and 4,000 mg L^{-1} IBA were applied, reaching a maximum survival of 93.53% after 42 days of acclimatization at the concentration of 2,925 mg L^{-1} IBA and maximum shoot length of 22.1 cm at the concentration of 2,863 mg L^{-1} IBA for the cultivar Columbus.

In general, the best results were obtained with the association between auxins with cytokinins, especially related to sprouting efficiency. This response occurred because organogenesis is dependent on the correct balance between auxins and cytokinins, and higher ratios between auxins and cytokinins induce higher root formation, low ratios induce shoot formation, and intermediate ratios induce callus formation (Takahashi, 2002). Thus, the studies with the most effective results were those whose balance between these plant regulators for *H. lupulus* L. was more adequate.

In contrast, no research was found showing which cultivars have the highest potential for endogenous auxin production,

which could result in alternatives for future studies with cytokinin application or even additional auxins applications.

Used plant organ

Table 3 shows that the best results considering sprouting efficiency were those that used young shoots as vegetative material, with an overall mean of 73.01% efficiency, reaching 96.6% in the treatment that used 0.57 µM IAA and 2.22 µM BA in an experiment conducted by Roy et al. (2001). The lowest results were obtained when using internodes, with a mean of only 36.2% efficiency. Importantly, this table lists only treatments with the best results from each manuscript. The other plant organs used as propagation material showed similar shooting efficiency results, with utilization close to 50% (46.67% for leaf, 45.80% for petioles, 48.9% for root tips, 51.10% for buds, and 44.70% for stem).

Effect on different cultivars

This survey found 50 cultivars used in the various studies, but the studies that compared the effects with different cultivars were developed by Batista et al. (1996), Sustar-Vozlic et al. (1999), Gurriarán et al. (1999), Reed (2005), Grudzińska et al. (2006), Skoff et al. (2007), and Yang et al. (2019), but the latter used unidentified accessions.

Batista et al. (1996) studied alternatives for in vitro propagation of the cultivars Bragança and Brewer's Gold and observed that Bragança had better regeneration and number of shoots under the tested conditions. The authors concluded that genotypic differences between cultivars can have significant effects on tissue culture response, demonstrating that although the genotype determines callus induction and plant regeneration, the influence of various conditions, such as culture medium composition, hormonal content, and the physiological state of the donor plant, depending on the cultivar, are fundamental in the behavior of in vitro cultures. Gurriarán et al. (1999) compared the cultivars Nugget and Brewer's Gold with different formulations of plant regulators and obtained higher means for the cultivar Nugget, reaching 50% rooting with a composition of 4.99 µM IBA and 4.40 µM BAP and 44% rooting with 4.99 µM IBA and 4.56 µM zeatin. The best result for the cultivar Brewer's Gold was 40% rooting with 4.99 µM IBA and 4.40 µM BAP.

Sustar (1999) worked with 16 cultivars and the best results for the number of shoots were for the cultivar Savinjski Golding, with 37.5% of shoots when using the combination of plant regulators [200/69 + kinetin (23.20) + IAA (0.29)].

Reed (2005) evaluated the cold storage time of in vitro culture of eight cultivars and obtained a longer storage time for the cultivar Arizona 1-2 (19 months). The best results for the percentage of regrowth when comparing 28 accessions were found for the accessions Arizona 1-2 and Cicero, both with a rate of 85%.

Grudzińska et al. (2006) evaluated the cold treatment and meristem excision time to eliminate hop latent viroid (HLVd) from four cultivars multiplied in vitro and concluded that the viroid elimination efficiency varied according to the hop cultivar, with the cultivar lunga showing 77% of free plants.

Skoff et al. (2007) evaluated three cultivars and obtained the best results with the cultivar Aurora compared to the regeneration of internode explants. The cultivar Savinjski Golding presented the best result for the variable tetraploid generation.

Effects of culture media

Most studies on the effects of culture media used the Murashige and Skoog (1962) medium (MS) in tests with modifications to some components and comparing it with other media (Figure 2). The variables number of shoots, number of calluses, number of roots, length of shoots and roots, as well as acclimatization and viability, were evaluated.

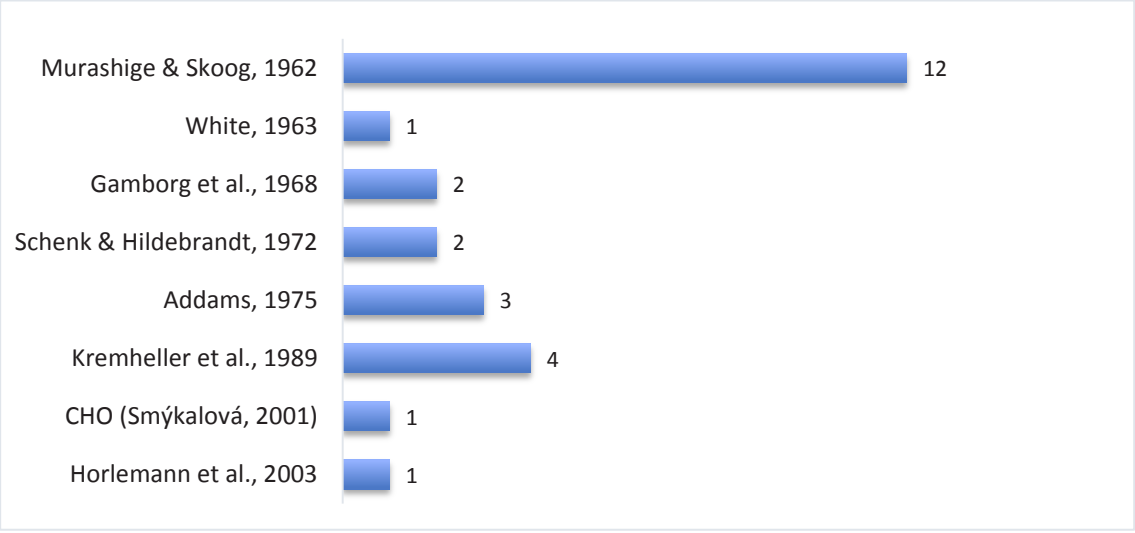

Figure 2. Main culture media used in studies involving micropropagation of *H. lupulus* L.

Among the studies, only that developed by Roy et al. (2001) showed better results with media other than MS or MS with some proposed modification (Table 4). The comparison between viability and efficiency in rooting and/or shoot formation showed that Smýkalová et al. (2001) had the best results using MS medium with half the concentrations of vitamins and salts, obtaining 100% formation of two shoots per explant. Machado et al. (2018) achieved a rate of 90.2% of callus formation with MS medium added with sucrose, myo-inositol, IAA, and TDZ.

Few studies compared the potential for vegetative propagation of different cultivars, and many of the main cultivars available on the market were not evaluated regarding the best technique for obtaining seedlings via micropropagation.

Table 4
Results of studies that compared different culture media for micropropagation of *H. lupulus* L

Culture medium	Best result	Reference
MS/HF + 0.1 µM NAA + 8.8 µM BAP	Number of roots (2.5 – 2.7)	Liberatore et al., 2020a
MS + 30 g L^{-1} sucrose + 100 mg L^{-1} myo-inositol + 7 g L^{-1} agar	Shoot length (27 mm)	Machado et al., 2018
MS + 20 g L^{-1} glucose + 100 mg L^{-1} myo-inositol + 4.4 mg L^{-1} BAP + 0.1 mg L^{-1} Ca pantothenate + 3 g L^{-1} agar + 1 g L^{-1} GelRite	Rooting rate (39.0%)	
MS + 30 g L^{-1} sucrose + 100 mg L^{-1} myo-inositol + 0.0547 mg L^{-1} IAA + 0.2189 mg L^{-1} TDZ + 7 g L^{-1} agar	Number of shoots (5.2), leaves (10.8), and callus rate (90.2%)	
White (1963)	Viability (53.9%)	Roy et al., 2001
MS + 10 µM IBA,10 µM BAP, 1 µM GA3	Viability (58.8%)	Svoboda, 1991
MS with ½ of the components and without growth regulators	Provided better rooting	
MS + 1 mg L^{-1} thiamine hydrochloride + 30 mg L^{-1} cysteine + 1.5% sucrose	Viability (72.2%)	Batista et al., 1996
CHO (MS with 1/2 concentration of vitamins and salts: 0.1 g dm3 myo-inositol + 15 g dm^3 glucose + 7 g dm^3 agar	Number of shoots (6) Efficiency in the formation of two shoots (100%)	Smýkalová et al., 2001
CHO (MS with 1/2 concentration of vitamins and salts: 0.1 g dm^3 myo-inositol + 40 g dm^3 maltose + 7 g dm^3 agar + 1 mm dm^3 zeatin + 0.1 mm dm^3 2,4-D	Frequency of calluses (70.8%)	

Obtaining virus-free plants

The first study available in the searched databases involving hop micropropagation techniques to obtain virus-free plants was published by Sallie and Jones (1969). Cultures were started from terminal shoots of hop plants with a size between 0.5 and 5.0 mm and culture medium supplemented with 1 mg L^{-1} of the sodium salt of gibberellic acid (GA_3), 1 mg L^{-1} of indole butyric acid (IBA), 0.2 mg L^{-1} of benzylaminopurine (BAP), and a mixture of vitamin B. An increase of about eight times the size of the shoots was observed in the period of 4-6 weeks. Only 1.4% of the obtained plants were infected by the HMV virus and 4.1% by the NRSV virus. Rooting was obtained by transferring the apical meristems to a medium without GA3. Subsequently, the virus detection test showed that three seedlings were infected with NRSV (prunus necrotic ringspot virus) and one with HLV (hop latent virus) among the 73 seedlings obtained by this process.

Oriniaková and Matousek (1996) investigated a way to avoid hop infection by HLV with transformation with the vector *Agrobacterium tumefaciens.* They achieved good results by changing the composition of the culture medium used in the micropropagation process by replacing ticarpen, widely used as an antimicrobial antibiotic, with augmentin (clavulanate-potentiated amoxicillin).

B. A. N. Adams et al. (1996) reported the importance of the tool cleaning process in crop management and at the time of collection of the vegetative material used in the micropropagation process. The authors also demonstrated that the material storage at a temperature of 2 °C for eight months showed 17 HLV-free materials among 20 tested. Grudzińska and Solarska (2005) performed in vitro propagation using meristems. Regenerated plants were tested by ELISA for the presence of viruses and by RT-PCR for the presence of HLVd. Plants without viruses and detected HLVd were used for further propagation. The efficiency of cold treatment of meristem culture to eliminate the hop latent viroid was also demonstrated by Grudzińska et al. (2006).

However, no studies related to the production of AHLV (American hop latent virus) and AMV (Alfalfa mosaic virus) free plants, two important viruses that cause diseases in hops.

Cryopreservation

Hop germplasm collections are commonly maintained in the field as perennial rhizomes. Diseases, insects, and environmental stresses put these plants at risk and viral diseases can accumulate in a field collection and be transferred to other locations by vegetative propagation (Reed, 2005).

Studies have been carried out since 1999 to develop and test techniques for cryopreservation of micropropagated material, aiming at the availability of pathogen-free material for the field planting period. Martinez et al. (1999) developed the first study using encapsulation in a medium with alginate and different concentrations of sucrose, followed by fast freezing and slow thawing. Shoot recovery after freezing for 60 minutes in liquid nitrogen was about 80%. Reed et al. (2003) adapted cryopreservation protocols developed for temperate climate cultures for *H. lupulus.* The authors evaluated the response of several genotypes to in vitro storage under low light at 4 °C following techniques used for strawberry and mint plants, and cryopreservation in liquid nitrogen by slow cooling with pear protocol. The mean storage time without transfer for the 70 evaluated genotypes was 14 months, with a range from 6 to 26 months. The mean recovery of cryopreserved meristems was 54% for accessions with two weeks of cold acclimatization.

Reed (2005) characterized the response of 70 genotypes to in vitro storage to cryopreservation in liquid nitrogen by slow cooling at 4 °C under low light conditions. The mean storage time without transfer for the evaluated genotypes was 14.1 months, with an interval of 6 to 26 months, a result very similar to the 2003 experiment. Seedlings for cryopreservation were cold acclimatized for 2 weeks, pretreated for 48 hours, cooled to −40 °C at 1 °C per minute, and immersed in liquid nitrogen. The mean recovery of cryopreserved tips of accessions was 54% after two weeks of cold acclimatization. Some genotypes required longer acclimatization for good recovery after exposure to liquid nitrogen. Liberatore et al. (2020a) also evaluated the encapsulation technique. Simple micro-cuttings were immersed in the encapsulating solution (AE enriched with sodium alginate at 2.5% (w/v) sodium salt of alginic acid) with medium viscosity and, subsequently, the alginate-coated propagules were immersed in

complexing solution (AE enriched with $CaCl_2$ at 1.1% w/v) for 35 min. All bare and encapsulated micro-cuttings kept their viability during the 4-week evaluation period.

Plant germplasm stored in vitro is usually evaluated by visual analysis based on subjective characters, which decreases accuracy. Aynalem et al. (2006) developed a digital image assessment system for *H. lupulus* L. stored in vitro to reduce variability in these assessments. The cultivars were stored in standard MS medium with iron (chelated EDTA) and MS medium with 100 or 200 mg L^{-1} of Sequestrene 138 iron (chelated EDDHA). Significant differences were observed between MNDVI (modified normalized difference vegetation index) values for seedlings stored in the medium with only standard MS iron (chelated EDTA) and the addition of Sequestrene 138 iron. In general, the MNDVI value of the top node correlated well with visual assessments and could be used to determine the health of in vitro stored hops.

Somaclonal variation

The exploitation of somaclonal variation may be a potential strategy to overcome the decrease in intraspecific hop biodiversity. Several strategies can be used to increase the induction of somaclonal variation, including differentiated explant material (leaves, roots, and stems), prolonged maintenance time of in vitro cultures, and a balanced relationship between auxins and cytokinins (Liberatore et al., 2020b).

Sustar-Vozlic et al. (1999) published a protocol for the regeneration of hop shoots evaluating regenerators for possible somaclonal variations that occur during the process of organogenesis from undifferentiated tissue, using RAPD markers and flow cytometric analysis. Genome size analysis by flow cytometry measurement of 46 regenerants revealed genome duplication to the tetraploid level in only four of the tested regenerants. Trojak-Goluch et al. (2015) estimated the effects of explant type and plant regulators on regeneration efficiency and polyploid induction.

The flow cytometry technique revealed that callus cultures older than 23 weeks led to DNA amplification and tetraploid formation among regenerated plants. The highest number of tetraploids (9.4%) was obtained from calluses derived from the petiole, and the number of polyploids was markedly increased by IAA addition to the regeneration medium.

Liberatore et al. (2020b) performed cytofluorimetric analysis and random amplification of polymorphic DNA (RAPD) to verify the occurrence of somaclonal variation. Mutants were detected among regenerants (16.8%) with more than half of the tetraploids obtained from the medium containing the highest BAP concentration (35.55 µM). Mutants detected by RAPD analysis were independent of medium composition and culture time. A strong influence on the explant was observed, and almost half of the obtained mutants originated from cultivated leaf tissues.

Other studied variables

In addition to the previously reported variables, studies have also evaluated the effects of artificial lighting and described the events of organogenesis. Fluorescent and incandescent bulbs are commonly used as

light sources when plants are propagated using tissue culture techniques, but they often present poor quality to promote proper seedling development. On the other hand, the use of LED lights can provide superior quality light emissions, such as red (660 nm) and blue (460 nm) wavelengths, which are enhanced for plant development. Moreover, the longevity and efficiency of LED lights make them ideal for micropropagation installations where supplemental lighting is used for long periods (Nguyen et al., 2020). The authors placed newly regenerated hop shoots of the cultivar Tettnanger under white light (WL) or LED light during 16 hours of light and 8 hours of dark at constant room temperature for four weeks and with and without charcoal in the culture medium. Hubbell's four-foot (62-watt) NutriLED unit LED lights produced a red spectral output of 660 nm, a blue spectral output of 460 nm, and a 2:1 red-to-blue LED ratio. Overall, the supplemental LED light promoted increases in both plant height, leaf area, and hop rooting in tissue culture on MS medium alone compared to media supplemented with white light. Although activated charcoal may be beneficial for the micropropagation of certain plant species, this component prevented growth under white light and LED light.

Fortes and Pais (2000) studied the sequence of histological and histochemical events that occur during the organogenesis of calluses derived from internodes. Cell division was observed in the cambial and cortical regions during the first week of establishment. Cell division in cortical cells led to the formation of incipient calluses from which pre-nodular structures of cambial origin emerged that gave rise to calluses that formed stem buds, which originated an increasing number of shoot buds. A higher amount of starch was found in explants grown in medium with plant regulators than in the control.

Conclusions

Regarding propagation by cuttings, few studies have shown that it is a species of easy rooting, even without the need to use plant regulators since percentages above 75% were obtained. On the other hand, there are gaps to be studied, such as cutting size, rooting time, viability between cuttings of main branches, secondary branches, or rhizome, and ease of rooting depending on the cultivar.

Regarding micropropagation, a lot of information was built, mainly relative to the use of plant regulators, with the best results by applying auxins and cytokinins, obtaining up to 96.6% of sprouting efficiency in culture media supplemented with 0.57 µM IAA and 2.22 µM BA and 90.8% in media with 2.85 µM IAA and 29.52 µM 2iP.

There was a trend of studies related to cryopreservation. This information is very important to the productive sector since the propagation material is currently taken only in the hot seasons, considering that the seedlings need to be ready to go to the field in early spring.

References

Adams, A. N. (1975). Elimination of viruses from the hop (Humulus lupulus) by heat therapy and meristem culture. *Journal of Horticultural Science, 50*(2), 151-160. doi: 10.1080/00221589.1975.11514616

Adams, B. A. N., Barbara, D. J., Morton, A., & Darby, P. (1996). The experimental transmission of hop latent viroid and its elimination by low temperature treatment and meristem culture. *Annals of Applied Biology, 128*(1), 37-44. doi: 10.1111/j.1744-7348.1996.tb07087.x

Alcantara-Cortes, J. S., Godoi, J. A., Cortés, J. D. A., & Mora, R. M. S. (2019). Principales reguladores hormonales y sus interacciones en el crecimiento vegetal. *Nova, 17*(32), 109-129. Retrieved from http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S1794-24702019000200109

Almaguer, C., Schönberger, C., Gastl, M., Arendt, E. K., & Becker, T. (2014). *Humulus lupulus* - a story that begs to be told, a review. *Journal of the Institute of Brewing, 120*(4), 289-314. doi: 10.1002/jib.160

Aynalem, H., Righetti, T., & Reed, B. M. (2006). Iron formulation affects in vitro storage of hops: an image analysis. *In Vitro Cellular & Developmental Biology - Plant, 42*(1), 405. doi: 10.1079/IVP2006798

Batista, D., Ascensão, L., Sousa, J. M., & Pais, M. S. (1999). Adventitious shoot mass production of hop (*Humulus lupulus* L.) var. Eroica in liquid medium from organogenic nodule cultures. *Plant Science, 151*(1), 47-57. doi: 10.1016/S0168-9452(99)00196-X

Batista, D., Sousa, M. J., & Pais, M. S. (1996). Plant regeneration from stem and petiole-derived callus of *Humulus lupulus* L. (Hop) clone Bragança and var. Brewer's Gold. *In Vitro Cellular & Developmental Biology - Plant, 32*(1), 37-41. Retrieved from https://www.jstor.org/stable/20064871

Behre, K. (1998). The history of beer additives in Europe - a review. *Vegetation History and Archaeobotany, 8*(1), 35-48. doi: 10.1007/BF02042841

Biendl, M., Forster, A., Schönberger, C., Engelhard, B., Gahr, A., Lutz, A.,... Schmidt, R. (2014). *Hops: their cultivation, composition and usage.* Nuremberger, Germany: Fachverlag Hans Carl.

Davis, T. D., Haissig, B. E., & Sankhla, N. (1988). Effect of shoot growth retardants and inhibitors on adventitious rooting. In T. D. Davis, B. E. Haissig, & N. Sankhla (Eds.), *Adventitious root formation in cuttings. Advances in plant sciences series* (pp.174-184). Portland, Ore: Dioscoride Press.

Denoma, J. S. (2000). *Humulus genetic resources: hop. USDA ARS National Clonal Germplasm repository.* Corvalis, Oregon, USA. USDA-ARS National Clonal Germplasm Repository. Retrieved from https://www.ars.usda.gov/pacific-west-area/corvallis-or/national-clonal-germplasm-repository/docs/ncgr-corvallis-humulus-germplasm

Durello, R. S., Silva, L. M., & Bogusz, S., Jr. (2019). Química do lúpulo. *Quimica Nova, 42*(8), 900-919. doi: 10.21577/0100-4042.20170412

Fachinello, J. C., Nachtigal, J. C., & Kersten, E. (2009). *Fruticultura: fundamentos e práticas.* Pelotas, RS, Brazil. Livro EMBRAPA Clima Temperado.

Fagherazzi, M. M., Santos, M. F., Santos, K. V., Rufato, L., & Moreira, M. A (2018). Propagação de estacas de lúpulo sob diferentes substratos. *Revista da 15.*

Jornada da Pós-Graduação e Pesquisa - Congrega Urcamp, 15(15), 1400-1409. Retrieved from http://revista.urcamp.tche.br/index.php/rcjpgp/article/view/2914/2023

Faltus, M., Bilavcik, A., Zamecnik, J., & Svoboda, P. (2007). Effect of phytohormone composition of nutrient medium on in vitro plant regeneration in hop clones with different sensitivities to indole-3-butyric acid. *Advances in Horticultural Science, 21*(1), 219-224. Retrieved from https://www.jstor.org/stable/42883538

Fortes, A. M., & Pais, M. S. (2000). Organogenesis from internode-derived nodules of Humulus Lupulus var. Nugget (cannabinaceae): histological studies and changes in the starch content. *American Journal of Botany, 87*(7), 971-979. doi: 10.2307/2656996

George, E. F., Hall, M. A., & Klerk, G. J. D. (2008). Plant growth regulators I: introduction; auxins, their analogues and inhibitors. In: E. F. George, M. A. Hall, & G. J. D. Klerk (Eds.), *Plant Propagation by Tissue Culture.* (pp.175-204). Springer, Dordrecht. doi: 10.1007/978-1-4020-5005-3_5

Gomes, E. N., Machado, M. P., Miola, J., & Deschamps, C. (2018). Leaf area and intermittent misting on hop plants propagation by stem cuttings. *Revista Colombiana de Ciências Hortícolas, 12*(2), 508-513. doi: 10.17584/rcch.2018v12i2.7652

Grudzińska, M., & Solarska, E. (2005). The elimination of viruses and hop latent viroid from hop (*Humulus lupulus* L.) in Poland. *Acta Horticulturae, 668*(1), 149-152. doi: 10.17660/ActaHortic.2005.668.19

Grudzińska, M., Solarska, A., Czubacka, A., Przybys, M., & Fajbus, A. (2006). Elimination of hop latent viroid from hop plants by cold treatment and meristem tip culture. *Phytopathological Polish, 40*(1), 21-30. Retrieved from http://www.up.poznan.pl/~ptfit1/pdf/PP40a/PP40_21-30.pdf

Guerreiro, D., & Reis, L. A. (2019). Avaliação da propagação de estacas de lúpulo (*Humulus lupulus* l.) submetidas à diferentes tipos de reguladores de crescimento. Anais do Enbralupulo - *Encontro Nacional de Pesquisadores e Produtores de Lúpulo,* Botucatu, SP, Brasil, 1.

Guimarães, J. J., Sousa, F. G. G., Sousa, R. L., Román, R. M. S., Sarnighausen, V. C. R., Dal Pai, A., & Rodrigues, S. A. (2019). Avaliação quantitativa da produção de mudas de lúpulo Cascade por estaquia e compostos orgânicos. *Anais do Enbralupulo - Encontro Nacional de Pesquisadores e Produtores de Lúpulo,* Botucatu, SP, Brasil, 1.

Gurriarán, M., Revill, M., & Tamés, R. (1999). Adventitious shoot regeneration in cultures of *Humulus lupulus* L. (hop) cvs. brewers gold and nugget. *Plant Cell Reports, 18*(1), 1007-1011. Retrieved from https://link.springer.com/article/10.1007/s002990050699

Hassanein, A. (2013). Fators influencing plant propagation efficiency via stem cuttings. *Journal of Horticultural Science & Ornamental Plants, 5*(3), 171-176. Retrieved from http://idosi.org/jhsop/5(3)13/ 4.pdf

Hinojosa, G. F. (2000). Auxinas. In L. P. B. Cid (Ed.), *Introdução aos hormônios vegetais* (pp. 15-54). Brasília: EMBRAPA.

Horlemann, C., Schwekendiek, A., Höhnle, M. & G. Weber (2003). Regeneration and agrobacterium-mediated transformation of hop (*Humulus lupulus* L.). *Plant Cell Reports, 22*(1), 210-217. doi: 10.1007/s00299-003-0676-8

Howard, B. H. (1965). Regeneration of the hop plant (*Humulus lupulus* L.) from softwood cuttings. I. the cutting and its rooting environment. *Journal of Horticultural Science, 40*(3), 181-191. doi: 10.1080/00221589.1967.11514198

Howard, B. H., & Sykes, J. T. (1966). Regeneration of the hop plant (*Humulus lupulus* L.) from softwood cuttings., *Journal of Horticultural Science, 41*(2), 155-163. doi: 10.1080/00221589.1966.11514164

Liberatore, C. M., Rodolfi, M., Beghè, D., Fabri, A., Ganino, T., & Chiancone, B. (2020a). Adventitious shoot organogenesis and encapsulation technology in hop (*Humulus lupulus* L.). *Scientia Horticulturae, 270*(1), 1-8. doi: 10.1016/j.scienta.2020.109416

Liberatore, C. M., Rodolfi, M., Beghè, D., Fabri, A., Ganino, T., & Chiancone, B. (2020b). In vitro leaf-derived organogenesis and somaclonal variant detection in *Humulus lupulus* L. *In Vitro Cellular & Developmental Biology - Plant, 56*(1), 865-874. doi: 10.1007/s11627-020-10088-7

Machado, M. P., Gomes, E. N., Francisco, F., Bernert, A. F., Bespalhok, J. C., Fº., & Deschamps, C. (2018). Micropropagation and establishment of *Humulus lupulus* L. plantlets under field conditions at Southern Brazil. *Journal of Agricultural Science, 10*(7), 275-281. doi: 10.5539/jas.v10n7p275

Martinez, D., Tamès, R. S., & Revilla, M. A. (1999). Cryopreservation of in vitro-grown shoot-tips of hop (*Humulus lupulus* L.) using encapsulation / dehydration. *Plant Cell Reports, 19*(1), 59-63, doi: 10.1007/s002990050710

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum, 15*(3), 473-497. doi: 10.1111/j.1399-3054.1962.tb08052.x

Neve, R. A. (1991). *Hops.* London: Chapman e Hall.

Nguyen, C. D., Vu, D., Huo, H., & Pearson, B. (2020). *LED Light increases leaf area and root length of Humulus lupulus (var. Tettnanger) in vitro.* Apopka, FL, USA. Ifas Extension, University of Florida. Retrieved from https://edis.ifas.ufl.edu/ep583

Nisler, J., Zatloukal, M., Popa, I., Doležal, K., Strnad, M., & Spíchal, L. (2010). Cytokinin receptor antagonists derived from 6-benzylaminopurine. *Phytochemistry, 717*(1), 823-830. doi: 10.1016/j.phytochem.2010. 01.018

Pasa, M. D. S., Carvalho, G. L., Schuch, M. W., Schmitz, J. D., Torchelsen, M. M., Nickel, G. K., & Camargo, S. S. (2012). Qualidade de luz e fitorreguladores na multiplicação e enraizamento in vitro da amoreira-preta 'Xavante'. *Ciência Rural, 42*(8), 1392-1396. doi: 10.1590/S0103-84782012000800010

Reed, B. M. (2005). In vitro storage of hop (*Humulus Lupulus* L.) germplasm. *Acta Horticulturae, 668*(1), 249-256. doi: 10.17660/ActaHortic.2005.668.32

Reed, B. M., Okut, N., Narver, L., & DeNoma, J. (2003). Cold storage and cryopreservation of hops (*Humulus lupulus*) shoot cultures through application of standard protocols. *CryoLetters, 24*(1), 389-396. Retrieved from https://pubmed.ncbi.nlm.nih.gov/14671691/

Robins, R. J., Furze, J. M., & Rhodes, M. J. C. (1985). Acid degradation by suspension culture cells of *Humulus lupulus. Phytochemistry, 24*(1), 709-714. doi: 10.1016/S0031-9422(00)84881-5

Roy, A. T., Leggett, G., & Koutoulis, A. (2001). Development of a shoot multiplication system for hop (*Humulus lupulus* L.). *In Vitro Cellular & Developmental Biology Plant, 37*(1), 79-83. Retrieved from https://www.jstor.org/stable/4293422

Sallie, J. V., & Jones, O. P. (1969). The culture of shoot tips of hop (*Humulus lupulus* L.) to eliminate viroses. *Journal of Horticultural Science, 44*(3), 281-284. doi: 10.1080/00221589.1969.11514310

Santos, M. F. S. (2019). Aclimatização de microestacas de diferentes cultivares de lúpulo (*Humulus lupulus* L.) Micropropagadas in vitro. *Anais do Simpósio Técnico-Científico da Cultura do Lúpulo e II Seminário Técnico da Aprolúpulo,* Botucatu, SP, Brasil.

Schwekendiek, A., Hanson, S. T., & Crain, M. (2009). A temporary immersion system for the effective shoot regeneration of hop. *Acta Horticculturae, 84*(1), 149-156. doi: 10.17660/ActaHortic.2009.848.16

Skof, S., Bohanec, B., Kastelec, D., & Luthar, Z. (2007). Spontaneous induction of tetraploidy in hop using adventitious shoot regeneration method. *Plant Breeding, 126*(1), 416-421. doi: 10.1111/j.1439-0523.20 07.01378.x

Smýkalová, I., Patzak, J., & Lipavská, H. (2001). Efficient in vitro micropropagation and regeneration of *Humulus lupulus* on low sugar. *Biologia Plantarum, 1*(1), 7-12. doi: 10.1023/A:1017901817063

Sommer, V. B., Fagherazzi, M. M., Santos, K. V. T., Fagherazzi, A. F., Muniz, A. T., Pierezan, H. C.,... Rufato, L. (2019). Aplicação de diferentes doses de Stimulate® para desenvolvimento radicular e vegetativo em mudas de lúpulo *(Humulus lupulus lupulus). Anais do Enbralupulo - Encontro Nacional de pesquisadores e produtores de lúpulo,* Botucatu, SP, Brasil, 1.

Sposito, M., Ismael, R., Barbosa, C. M. A., & Tagliaferro, A. (2019). *A cultura do lúpulo.* Piracicaba: ESALQ. (Série Produtor Rural). Retrieved from https://www.esalq.usp.br/biblioteca/content/68-cultura-do-l%C3%BApulo

Sustar-Vozlic, J., Javornik, B., & Bohanec, B. (1999). Studies of somaclonal variation in hop (*Humulus lupulus* L.). *Phyton, Special issue: Plant Physiology, 39*(3), 283-287. Retrieved from https://www.zobodat.at/pdf/PHY_39_3_0283-0287.pdf

Svoboda, P. (1988). Hormonal regulation of the growth of isolated apical meristems of hop (*Humulus lupulus* L.) *in vitro. Plant Production Science, 34*(1), 713-716. Retrieved from https://eurekamag.com/research/001/609/001609292.php

Svoboda, P. (1991). Clonal propagation hops in vitro. *Plant Soil and Environment, 37*(1), 643-648. Retrieved from https://www.researchgate.net/publication/266203478_Clonal_propagation%20_hop_in%20_vitro

Svoboda, P. (1992a). Hop callus culture. *Plant Soil and Environment, 38*(1), 961-965. Retrieved from https:// www.researchgate.net/publication/266478135_Hop_callus_culture

Svoboda, P. (1992b.) Hop explantate cultures. *Plant Soil and Environment, 38*(1), 107-112. Retrieved from https://www.researchgate.net/publication/264533679_Hop_explantate_cultures

Svoboda, P. (1992c). Cultivation of isolated hop tops (*Humulus Lupulus* L) *in vitro. Rostlinna Vyroba, 38*(6), 523-528. Retrieved from https://www.researchgate.net/publication%20/266396281_Cultivation_of_isolated_%20hop_%20tips_Humulus_lupulus_L_in_vitro

Svoboda, P. (1995). The effect of some regulators on the grow th of isolated top meristems of hops (*Humulus lupulus* L.) in in vitro culture. *Plant Soil and Environment, 41*(1), 379-381. Retrieved from https://www.researchgate.net/publication/264234734_the_effect_of_some_regulators_on_the_grow_th_of_isolated_top_meristems_of_hops_humulus-lupulus_l_in_in-vitro_culture

Takahashi, E. K. (2002). *Transferência do gene atacina A para plantas de maracujá amarelo (Passiflora edulis Sims f. flavicarpa Deg.) por biobalística.* Tese de doutorado em Engenharia Agrícola, Universidade de São Paulo, Piracicaba, SP, Brasil.

Taniguchi, Y., Taniguchi, H., Yamada, M., Matsukura, Y., Koizumi, H., Furihata, K., & Shindo, K. (2014). Analysis of the components of hard resin in hops (*Humulus lupulus* L.) and structural elucidation of their transformation products formed during the brewing process. *Journal of Agricultural and Food Chemistry, 62*(47), 11602-11612. doi: 10.1021/jf504394h

Trojak-Goluch, A., Kawka, M., & Czarnecka, D. (2015). The effects of explant source and hormone content on plant regeneration and induction of tetraploids in *Humulus lupulus* L.. *In Vitro Cellular & Developmental Biology - Plant, 51*(1), 152-159. doi: 10.1007/s11627-014-9661-x

White, P. R. (1963). *The cultivation of animal and plant cells.* New York: Ronald Press. Retrieved from https://science.sciencemag.org/content/141/3580/515.1

Yang, K., Chen, Y. Y., & Wang, J. C. (2019). Selection of different explants from hops and establishment of a regeneration system. *Acta Prataculturae Sinica, 28*(8), 209-217. doi: 10.11686/cyxb2019160