11-2017

Challenges towards Revitalizing Hemp: A Multifaceted Crop

Craig M. Schluttenhofer
University of Kentucky, craig.schluttenhofer@uky.edu

Ling Yuan
University of Kentucky, lyuan3@uky.edu

Challenges towards Revitalizing Hemp: A Multifaceted Crop

Notes/Citation Information

Published in *Trends in Plant Science*, v. 22, issue 11, p. 917-929.

Digital Object Identifier (DOI)

https://doi.org/10.1016/j.tplants.2017.08.004

Cell Press REVIEWS

Opinion

Challenges towards Revitalizing Hemp: A Multifaceted Crop

Craig Schluttenhofer[1,2] and Ling Yuan[1,2,3,*]

Hemp has been an important crop throughout human history for food, fiber, and medicine. Despite significant progress made by the international research community, the basic biology of hemp plants remains insufficiently understood. Clear objectives are needed to guide future research. As a semi-domesticated plant, hemp has many desirable traits that require improvement, including eliminating seed shattering, enhancing the quantity and quality of stem fiber, and increasing the accumulation of phytocannabinoids. Methods to manipulate the sex of hemp plants will also be important for optimizing yields of seed, fiber, and cannabinoids. Currently, research into trait improvement is hindered by the lack of molecular techniques adapted to hemp. Here we review how addressing these limitations will help advance our knowledge of plant biology and enable us to fully domesticate and maximize the agronomic potential of this promising crop.

Trends

For states which define hemp (<0.3% THC) as distinct from marijuana, the USA Agriculture Act of 2014 allows departments of agriculture or universities to cultivate hemp as part of a research pilot program.

As of 2017, at least 39 US universities and dozens of researchers have begun studying hemp, yet guidance on top research priorities are lacking.

While traditionally a fiber and grain crop, hemp has emerged as a source of nonhallucinogenic medicinal phytocannabinoids (e.g., CBD) with distinct properties from marijuana. Dozens of clinical studies are now investigating anecdotal uses of CBD to treat various medical conditions.

The last several years have seen advancements in understanding Cannabis genetics through publications of a draft genome, transcriptome sequencing, quantitative trait mapping, and genetic comparisons between hemp and marijuana.

Hemp: A Multifaceted and Diverse Plant

The genus *Cannabis* (commonly classified into the species *Cannabis indica*, *Cannabis sativa*, and *Cannabis ruderalis*) has been used for food, fiber, and medicine for over six millennia [1,2]. Depending upon the use, Cannabis is organized into two distinct groups – marijuana and hemp. Marijuana, primarily used recreationally for its intoxicating properties, may have medicinal value [3–5]. In contrast, hemp is valued for its medicinal compounds, fiber, and seed that are collectively used in over 25 000 known products [6]. Compared to marijuana, the medicinal compounds of interest found in hemp are nonintoxicating, for example, cannabidiol (CBD). In European and North American countries, to be legally classified as hemp the crop may not contain more than 0.2% or 0.3% of the intoxicating compound Δ^9-tetrahydrocannabinol (THC), respectively. This level of THC in Cannabis is insufficient to induce intoxication. Differences in cultural practices of marijuana and hemp result in minor variations in morphologies, allowing some distinction between these two crops [6].

Traditionally, hemp is grown for either seed or fiber. Hemp seeds contain approximately 30% protein, 25% starch, and 30% oil [7,8]. Pressed seeds release an oil that contains >90% polyunsaturated fatty acids. With a desirable ratio of ω-6 to ω-3 lipids [7,8], hemp seed oil is a valuable addition to human and animal diets [9]. Additionally, the oil can be used for cooking or processed into cosmetics and fuels [10,11]. The residual seed cake can be used for protein rich animal feed. **Bast fibers** (see Glossary) are primarily used to make high quality papers, whereas most **hurd** goes into animal beddings [10]. Recent technological advances have expanded the use of hemp fiber and hurd to include the production of carbon nanosheets, plastics, 3D-printer filaments, oil absorbent materials, and construction concrete. Additionally, hemp produces over 100 known cannabinoids, most notably CBD [12]. In the USA, clinical

[1]Department of Plant and Soil Sciences, University of Kentucky, Lexington, KY 40546, USA
[2]The Kentucky Tobacco Research and Development Center, University of Kentucky, Lexington, KY 40546, USA
[3]South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, China

*Correspondence: lyuan3@uky.edu (L. Yuan).

Cell Press REVIEWS

trials are investigating CBD for treatment of 26 medical conditions[i] (Table 1). Furthermore, CBD has been granted **orphan drug status** for eleven conditions (Table 1).

Recently, hemp production has expanded beyond Eurasia and Canada to include three additional countries: Greece, Malawi, and the USA (Figure 1A). The rapid expansion in the USA may considerably impact the global hemp market. Several recent reviews have provided detailed information on the biochemistry, breeding, ecology, genetics, morphology, pathology, physiology, and production of Cannabis [13–23]. Despite recent progress, much remains to be

Table 1. Ongoing USA Clinical Trials and Orphan Medical Conditions for Which CBD Has Been Approved

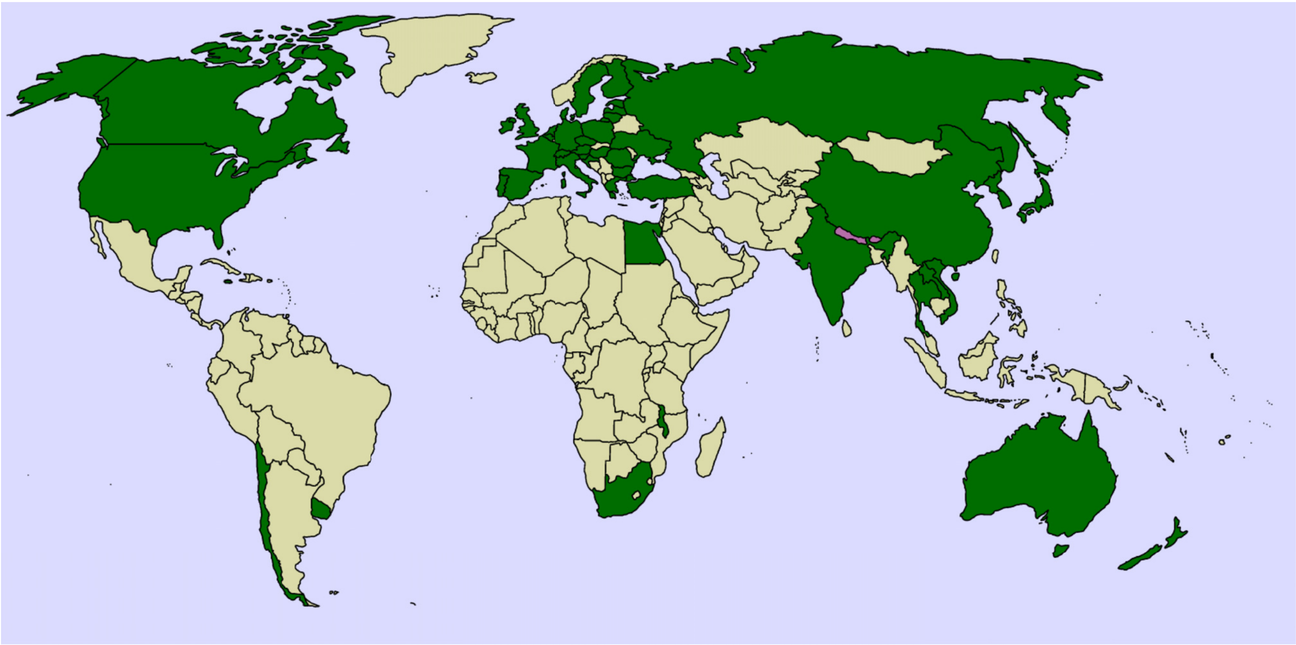
Medical condition[a]	Phase I	Phase II	Phase III	Orphan drug designation[b]
Amphetamine addiction	n.d.	Yes	n.d.	n.d.
Anxiety	n.d.	Yes	n.d.	n.d.
Autistic disorder	n.d.	Yes	n.d.	n.d.
Cannabis use disorder	n.d.	Yes	n.d.	n.d.
Cocaine dependence	n.d.	Yes	n.d.	n.d.
Dravet syndrome	n.d.	n.d.	Yes	Designated
Drug-resistant epilepsy	Yes	Yes	n.d.	n.d.
Epileptic encephalopathy	Yes	n.d.	n.d.	n.d.
Fatty liver	n.d.	Yes	n.d.	n.d.
Fragile X syndrome	n.d.	n.d.	n.d.	Designated
Glioblastoma multiforme	Yes	n.d.	n.d.	Designated
Glioma	n.d.	n.d.	n.d.	Designated
Graft versus host disease	Yes	Yes	n.d.	Designated
Graft versus host disease – prevention	n.d.	n.d.	n.d.	Designated
Infantile spasms	n.d.	Yes	Yes	Designated
Lennox–Gastaut syndrome	n.d.	n.d.	Yes	Designated
Neonatal hypoxic ischemic encephalopathy	n.d.	n.d.	n.d.	Designated
Lung cancer	Yes	n.d.	n.d.	n.d.
Opiate addiction	Yes	Yes	n.d.	n.d.
Pain	Yes	Yes	Yes	n.d.
Parkinson disease	n.d.	Yes	n.d.	n.d.
Pediatric intractable epilepsy	Yes	Yes	n.d.	n.d.
Pediatric schizophrenia	n.d.	n.d.	n.d.	Designated
Posttraumatic stress disorder	n.d.	Yes	n.d.	n.d.
Prader–Willi Syndrome	n.d.	Yes	n.d.	n.d.
Schizophrenia	Yes	Yes	n.d.	n.d.
Solid tumor	n.d.	Yes	n.d.	n.d.
Sturge–Weber Syndrome	Yes	Yes	n.d.	n.d.
Treatment-resistant seizures	Yes	n.d.	Yes	n.d.
Tuberous sclerosis complex	n.d.	n.d.	Yes	Designated
Ulcerative colitis	Yes	Yes	n.d.	n.d.

Abbreviations;: n.d., no data.
[a]Current as of February 16th, 2017 and considers CBD-only or high CBD/low THC formulations.
[b]Orphan drug designation.

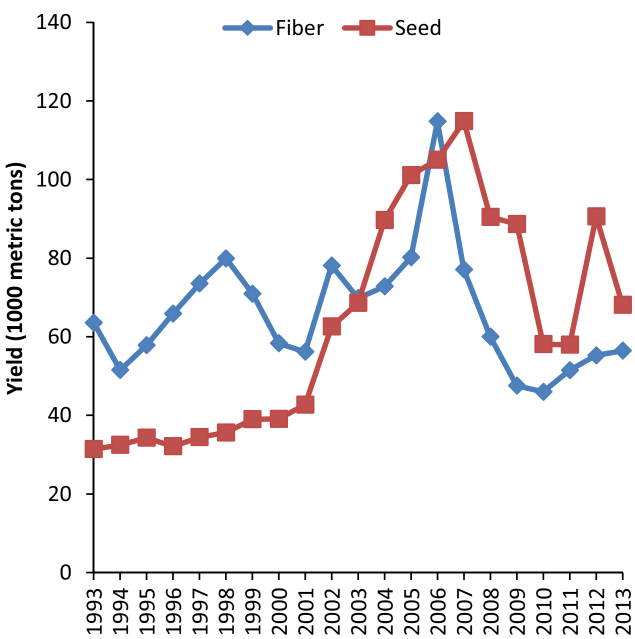
Glossary

Anemophilous: wind-pollinated.
Autoflowering: when flowering occurs independently of the photoperiod.
Bast fiber: long cellulose-rich bundles of phloem cells located underneath the stem epidermis.
Dioecious: male and female flowers are found on separate plants.
Female predominant: a population of consisting of 70–95% female plants and with the remaining plants being male or monoecious.
Hurd: the inner woody core of hemp stems.
Monoecious: separate male and female flowers are found on the same plant.
Orphan drug status: a special designation given by the USA Food and Drug Administration to a drug being investigated to treat a rare disease or disorder that affects less than 200 000 people in the USA, or for larger populations where investments are not expected to be recovered.
Shattering: the loss of mature seeds from the inflorescence.

(A)



(B)



(C)

Country	Fiber (ha)	Seed (ha)	Total ha†
Austria	600	–	600
Canada*	n/a	n/a	34262
Chile	4500	2200	6700
China	5000	5500	10500
Czech Republic	200	–	200
France	600	7706	8306
Hungary	300	1400	1700
Italy	425	–	425
Netherlands	1284	–	1284
North Korea	20000	–	20000
Poland	70	60	130
Romania	1600	1440	3040
Russia	4000	1644	5644
South Korea	12	–	12
Spain	10	2	12
Turkey	20	1	21
Ukraine	2000	2100	4100
Total	40621	22053	90906

Trends in Plant Science

Figure 1. Global Production of Hemp Fiber and Seeds. (A) Countries cultivating (green) or utilizing natural hemp populations for textiles (purple; Nepal and Bhutan). (B) Global production of hemp for fiber and seed from 1993 to 2013. (C) Countries producing hemp. Data was collected from the FAO for the year 2013. Total land area may be smaller depending on the quantity of dual-purpose (i.e., cultivars used for seed and fiber production) hemp planted. Annual statistics are from the FAO. Abbreviation: ha, hectares. *Value is from Health Canada for the year 2015. †Total production area assumes a single-purpose crop.

learned about this multifaceted and diverse plant. Importantly, there is limited information available about key research challenges that need addressed to improve this valuable crop. Thus, our objectives are (i) to briefly highlight the renewed interest in hemp, and (ii) illustrate strategic issues that should be addressed by researchers. While we focus on traits to improve hemp yield, these target research topics, in the long term, will also reveal important information about basic plant biology and domestication.

The Global Hemp Market

Viewed as an eco-friendly and highly sustainable crop [24], the global market for hemp is predicted to double from the year 2016 to 2020. At present, hemp is cultivated for commercial or research purposes in at least 47 countries, and it is utilized by indigenous populations for textiles in another two countries (Figure 1A). Since 2011, there has been an increase in hemp tonnage and acreage worldwide (Figures 1B and 1C). Statistics for hemp production are available from the Food and Agriculture Organization of the United Nations (FAO) for 16 countries (Figure 1C). Canada, China, Chile, France, and North Korea are currently the largest producers of hemp.

The USA is the largest importer of hemp products [6], obtaining most of its seed and fiber from Canada and China, respectively. In the Agricultural Act of 2014, the USA government authorized research into industrial hemp production (Figure 2A). Consequently, hemp production and research have rapidly increased in multiple states (Figure 2B). Establishment of a USA hemp industry may impact global commerce by reducing hemp imports from exporting countries.

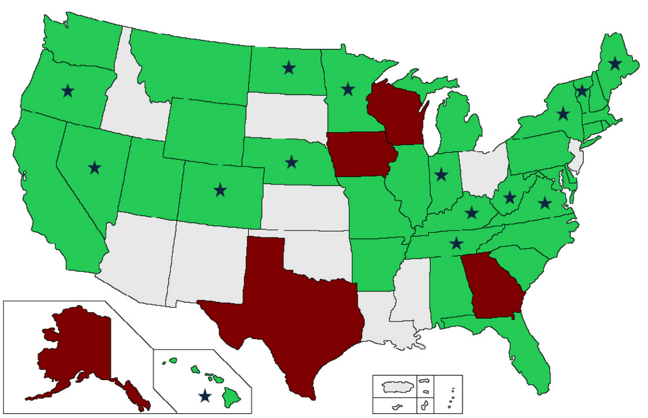
As consumer demand for organic and environmentally sustainable products increases, there is a potential for significant growth of the world hemp market. Currently, there is a major discrepancy in crop value depending on product type; for example, the value of CBD far exceeds that of seed or fiber (Figure 2C). Using 2015 market prices and excluding costs [25], revenue/ha is estimated to range from $625–$25 000 (Figure 2C). To advance the industry, a focus on developing or improving products that can penetrate multibillion dollar markets (e.g., livestock health, improved construction materials, or energy storage) should be encouraged. Increasing demand for hemp-derived products will help solidify a long-term sustainable market.

Future Directions for Hemp Research

Hemp is a genetically diverse and variable crop that produces raw products in three distinct categories: seed/oil, fiber, and metabolites. Within each category, hemp can be improved by multiple avenues of research. We highlight key research areas which increase grower yield or product quality for processors. These topics are not exhaustive, but are intended to guide research to areas which are of the highest priority.

Notably, due to the diverse nature of raw products produced from hemp, research targeting hemp yield traits will improve our understanding of basic plant biology. Seed and oil research will enrich our comprehension of grain yield and composition. Research into hemp fiber will enhance our knowledge of stem development and composition, genetic regulation of fiber traits, and biofuel production. Studies targeting metabolite yield will expand insights into both Cannabis-specific and shared plant chemistries, interaction with biotic stresses, and trichome development. Investigations into the plasticity of hemp’s sexual phenotype will contribute to identifying mechanisms underlying plant sex determination. Importantly, unlike previously domesticated crops [26,27], selection for increased hemp yield provides a unique opportunity to study plant domestication for grain, fiber, and chemistry traits. With hemp, unlike most other crops, these valuable characteristics can be studied within a single species for which they are essential to sustainable and profitable production.

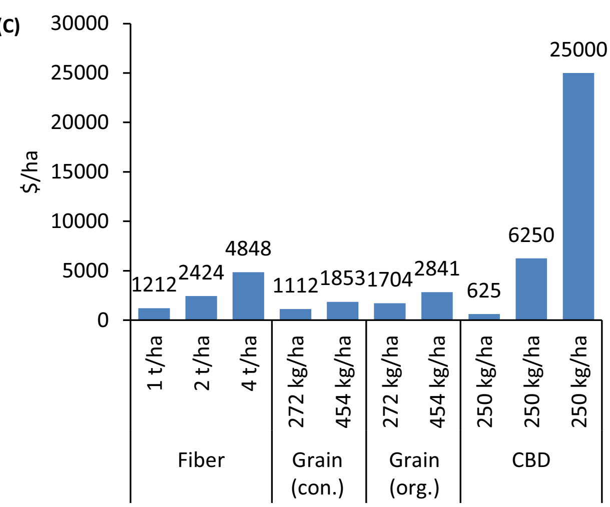
(A)



(B)

State	Year	Area planted (ha)	# Universities
Kentucky	2014	13.35	6
	2015	373.12	8
	2016	1021.83	10
Colorado	2015	930.78	1
	2016	2396.35	2
Hawaii	2015	0.10	1
	2016	0.00	1
Indiana	2015	1.62	1
	2016	0.81	1
Maine	2016	0.40	0
Minnesota	2016	20.64	1
Nebraska	2016	0.00	1
Nevada	2016	87.53	1
New York	2016	12.14	3
North Dakota	2015	0.08	1
	2016	28.33	1
Oregon	2015	20.23	1
	2016	202.34	1
Tennessee	2015	283.28	2
	2016	91.05	2
Vermont	2015	8.09	1
	2016	24.28	1
Virginia	2016	14.97	3
West Virginia	2016	4.05	1
Total	**2014**	**13.35**	**6**
Total	**2015**	**1617.31**	**16**
Total	**2016**	**3904.73**	**29**

(C)



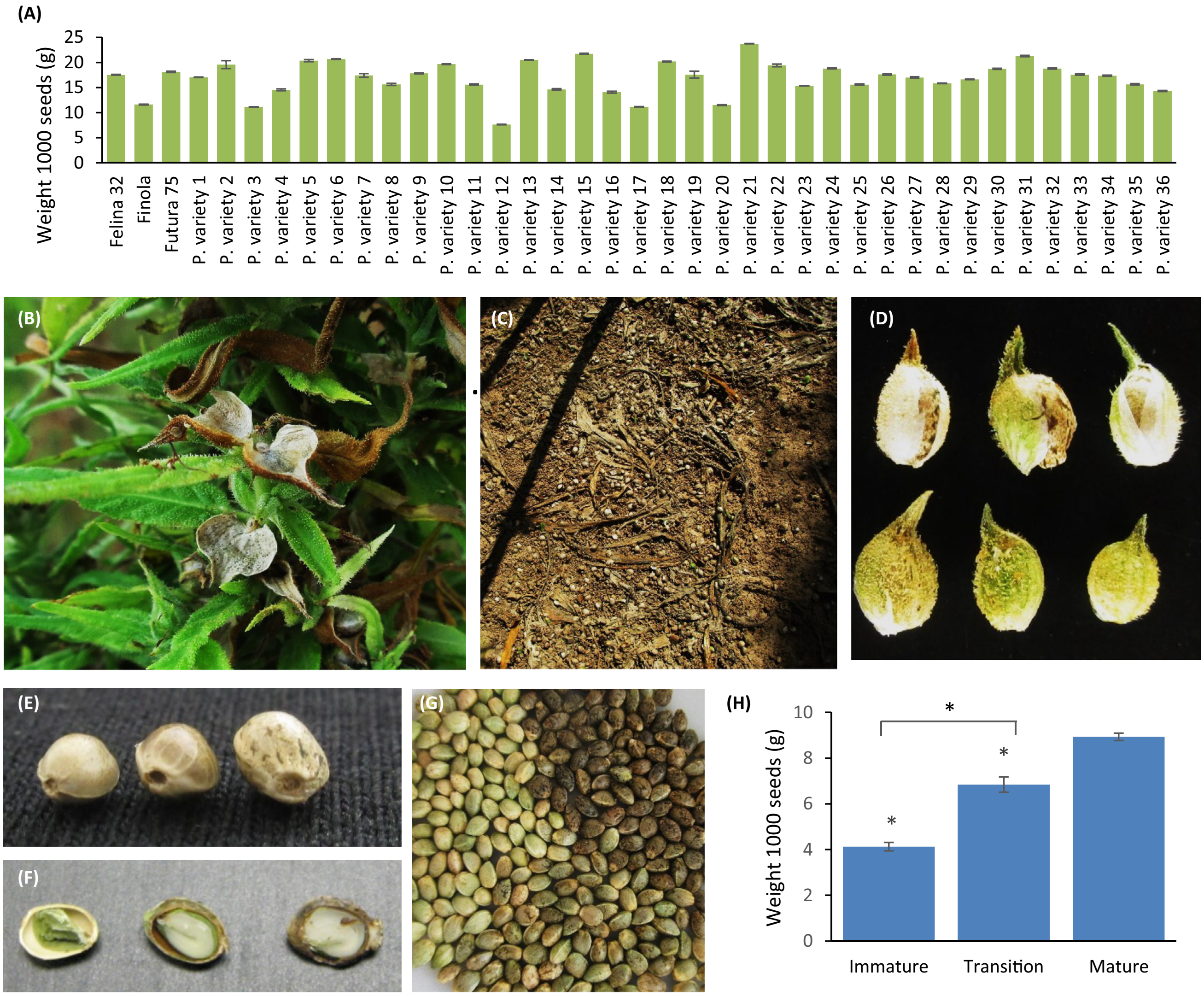
Trends in Plant Science

Figure 2. Hemp Production in the USA. (A) USA states currently able (green) and those pursuing legislation (red) to grow hemp, according to the 2014 Farm Bill. States that conducted hemp trials in 2016 are denoted with a blue star. In 2014, ten states (California, Colorado, Kentucky, Maine, Montana, North Dakota, Oregon, Vermont, Washington, and West Virginia) had in practice the necessary distinctions between marijuana and hemp, qualifying them for hemp research. Currently, 33 states meet the qualifying criteria. (B) USA states growing hemp, number of hectares planted, and the number of participating universities and colleges. Data obtained from VoteHemp[iv] and state Departments of Agriculture. (C) Revenue of hemp fiber, grain (conventional and organic), and phytocannabinoid (CBD) products per hectare. Prices are based on those paid during 2015. Fiber values assume a price of $1.21/kg of actual fiber. Assuming bast fibers make up 25% of the stem dry matter, then 1, 2, and 4 metric ton/ha are equal to 4, 8, and 16 metric ton/ha of stems, respectively. Grain production assumes a price of $1.65 and $2.54 per kilogram of conventional conventional (con.) or organic (org.) seed, respectively. Phytocannabinoid prices were obtained from hemp producers and CBD processors. CBD prices are complex, ranging from $2.50 to 10.00/g of pure CBD. Higher prices are paid for crops with higher percentages of CBD in flower material. Here, revenue per hectare was calculated using $2.50/g for 1%, $5.00 for 5%, and $10.00 for 10% CBD concentration in flower material.

Grain and Oil Production

As a semi-domesticated crop [28], many traits for hemp seed and oil yield require improvement; these include seed size consistency and improved **shattering** resistance. Significant advances in hemp seed production occurred with the development of FIN-314 (‘Finola’), an **autoflowering** grain variety with a short stature, adaptation to high latitudes, and high yield [29], resulting in it presently being the most popular cultivar grown in Canada [30]. However, seed size is highly variable among hemp cultivars (Figure 3A) [16] and ‘Finola’ seeds are ~50% the size of many commercial varieties (Figure 3A). Selection for genetically stable cultivars with larger seeds will be important for increasing hemp grain yields.

Cell Press REVIEWS



(I)

Crop	Biomass† (Mg DM/ha/y)	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Refs
Hemp fiber	7–34.0‡	73–77	7–9	2–6	[47–50]
Hemp hurd		34–48	21–25	17–19	[48]
Corn stover	4.6–5.5	38	26	17	[51,52]
Switchgrass	5.4–34.6	28–37	23–27	15–18	[53,54]
Giant *miscanthus*	10.0–44.0	50–52	25–26	12–13	[55]
Poplar	7.7–17.3	42–49	16–24	21–30	[56]
Willow	6.2–21.5	46–56	12–14	13–14	[57,58]

Trends in Plant Science

Figure 3. Properties of Hemp Seeds and Biomass. (A) 1000-seed weights for 39 hemp varieties. 'P. variety' designates a proprietary variety. (B) Seeds remain weakly attached to the plant but are susceptible to loss due to shattering. (C) Seeds shattered onto the ground prior to harvesting. (D) Seeds still partially (top) or completely (bottom) encased in bracts and perianth. (E) Hemp seeds showing the point of disarticulation at the base. (F) Dissected immature, transitional, and mature seeds (left to right) showing stages of seed fill. (G) Counterclockwise from upper left: immature, transitional, and mature hemp seeds. Seeds at each stage of

(See figure legend on the bottom of the next page.)

During domestication, hemp has retained little resistance to shattering [14]. However, hemp field trials have revealed that significant grain is lost due to shattering prior to and during harvesting as a result of inconsistent inflorescence maturity (Figures 3B and 3C), particularly if collected outside of the optimal harvest time windows. To mitigate this problem, growers harvest seeds at 70% maturity[ii] [31]. Hemp inflorescences are large multi-seeded heads in which each individual seed is partially surrounded by a bract, and an abscission zone connects the hull to the pedicle (Figures 3D and 3E). Selection for a stronger-walled abscission zone or the prevention of bracts releasing seeds (Figures 3D and 3E) are possible physiological traits to target to reduce hemp seed loss due to shattering. Furthermore, immature seeds are similar in size, but weighed only half that of mature seeds due to incomplete embryo development (Figures 3F, 3G and 3H). Without shattering, immature seeds would all fully mature, increasing yield by up to 15%. Thus, further domestication of non-shattering cultivars could greatly improve yield via a multifold mechanism, preventing harvesting loss and permitting all seeds to reach maturity.

Seed traits that expand market options will also be valuable. For example, there has been little research investigating the differences in hemp seed flavors. Taste tests in our lab identified varieties with weak to strong flavors of hazelnut (cv. 'Georgina') or walnut (cv. 'CRS-1'), as well as one (cv. 'Victoria') with a mild flavor. More work has been done on altering seed oil composition [32], although hemp seeds already possess valuable ω-3 characteristics [7]. Hemp seed oil is ~85% polyunsaturated fatty acids with 60% and 24% being ω-6 and ω-3 fats, respectively [7]. Further increases in ω-3 fatty acid levels might foster the favorability of hemp seed for human and animal dietary needs. Overall, different tastes and oil compositions would expand the use of hemp seed in human and animal food products.

Production and Quality of Hemp Fiber

Hemp stalks contain two key fractions, the bast fiber and hurd. To separate bast fibers from the inner hurd, the stalks must undergo a process called 'retting'. Retting relies on the diverse microbial populations in the environment to break down pectin and other components that bind the fibers to the hurd tissue [33,34]. Crop maturity at harvest, retting method, environmental conditions, as well as the nature of the bacterial and fungal populations, are factors that impact retting [35–37]. Harvesting the crop at initiation of flowering improves fiber yield, strength, and quality [33,38,39]. Continuing studies on the biodiversity, relationships, and functions of microbial communities will improve our understanding of the retting process [40–43] and augment the consistency of obtaining high quality products. Retting methods, primarily dew- and water-retting, pose drawbacks, including inconsistent fiber strength and quality, and polluted wastewater, respectively [34]. Development of varieties having bast fiber with higher cellulose content as well as lower pectin and lignin cross-linkages may decrease the retting requirements, thus improving fiber strength and quality while saving time and labor.

Hemp is a rapidly growing plant that tolerates high planting density [30,44–47], and may therefore be suitable as a viable biofuel crop. The total biomass of hemp per hectare is similar to other energy crops, including giant miscanthus (*Miscanthus* × *giganteus*), poplar (*Populus* sp.), switchgrass (*Panicum virgatum*), and willow (*Salix* sp.) (Figure 3I). However, hemp may provide a key advantage; its bast fibers contain 73–77% cellulose, 7–9% hemicelluloses, and 2–6% lignin, compared to 48%, 21–25%, and 17–19%, respectively, in the hurd [48–50]. Thus, the

development are similar in size. (H) 1000-seed weights of immature, transitional, and mature seeds of the variety Big Al Kentucky Plume. *, p-values of t-test <0.001. (I) Fiber yield and composition of hemp compared to other proposed biomass crops. Also see [47–58]. †Weight of dry matter (DM) includes moisture content at time of harvest. ‡Biomass is for fiber and hurd combined. Typically, stem material is 20–30% fiber.
For a Figure360 author presentation of Figure 3, see the figure online at http://dx.doi.org/10.1016/j.tplants.2017.08.004#mmc1

concentration of digestible cellulose and hemicellulose is higher in hemp fiber than in other energy crops (Figure 3I) [51–58]. In contrast, the cellulose and hemicellulose contents of hemp hurd are comparable to that in stems of giant miscanthus, poplar, switch grass, and willow. Importantly, 20–30% of the stem biomass in hemp consists of high cellulose fiber; thus, the ratio of digestible sugars to lignin is higher in hemp than in other similar-yielding biofuel crops. These traits make hemp an above-average energy crop for some biochemical-based biofuel production and greenhouse gas abatement applications [59,60]. Establishment of hemp as a biofuel crop would be beneficial to the industry by increasing demand for hurd and fiber.

Phytocannabinoids and Other Metabolites

Hemp produces a diverse array of nonintoxicating phytocannabinoids, terpenes, and phenolic compounds with potential pharmaceutical values as drugs or supplements [3,61,62]. The biosynthesis of terpenophenolic phytocannabinoids in Cannabis is well understood, albeit, several early steps in the pathway remain to be characterized [63,64]. Understanding the regulation of phytocannabinoid biosynthesis is vital to development of varieties that are optimized for production of desirable metabolites while maintaining low levels of THC. Little is known about the endogenous and environmental regulation of phytocannabinoids. Abscisic acid, ethylene, and gibberellic acid modulate the production of phytocannabinoids [65–67]. However; at present, factors controlling the epigenetic, transcriptional, and post-transcriptional regulation of phytocannabinoid biosynthesis remain uncharacterized.

Hemp trichomes are classified into bulbous, capitate-sessile, capitate-stalked, and nonglandular types [13,68]. Phytocannabinoid production and accumulation are localized to the capitate-stalked glandular trichomes [13,69]. Increased production of phytocannabinoids in marijuana is, at least partially, due to the presence of larger glandular trichomes [70]. Elucidating hormonal and other signaling cascades that regulate the development and size of specific types of hemp trichomes will also be important in maximizing phytocannabinoid production in hemp.

The effects of agronomic practices and nutrients on phytocannabinoid production also need to be investigated. Anecdotal claims from marijuana growers suggest that pollination of Cannabis flowers lowers phytocannabinoid yield [71], consistent with decreases in essential oil levels [72]. Further studies to evaluate this concern are essential to maximize the production of CBD and other desired phytocannabinoids.

Hemp Breeding Limitations

Germplasm collections are a fundamental source of genetic and phenotypic diversity for plant breeding and research. Currently, access to and utility of accession collections remain limited due to the lack of a core Cannabis germplasm collection. As THC levels may limit germplasm utility in many regions, accessions with $<0.3\%$ THC should be used to form a hemp-only germplasm core collection. Establishment of a core collection encompassing the range of hemp genetic and phenotypic diversity would increase the utility of germplasm resources and be invaluable for breeding and genetic analyses. Comparisons of accessions present in existing collections [73] are needed to help establish such a core collection. Similarly, centralized and curated collections of hemp mutants are not available. The development of mutant germplasm collections will provide a rich source of genetic variation for studying gene function and improved traits for breeding.

Hemp is an **anemophilous** crop in which the pollen can travel long distances. Studies in southern Spain identified Cannabis pollen in atmospheric samples which arrived from the extensive marijuana fields in Morocco over 100 km away [74,75]. Long-distance pollen dispersal causes difficulty for breeding programs which require spatial or mechanical methods for germplasm isolation. Cost-effective and efficient methods are needed that will allow breeders to develop multiple new hemp varieties simultaneously in a limited growing area.

Hemp Sex Expression

Hemp is a **dioecious** plant with female and male hemp plants being valued differently depending upon the products. For phytocannabinoid production, a pure female population is most desirable. As a seed crop, a **female predominant** population, with a limited number of male plants for pollination, or a **monoecious** variety, is most desirable to maximize yield. For fiber production, males and females are both utilized, although males are preferred [15,18]. Therefore, a major goal of hemp growers and breeders is to quickly and easily determine or manipulate the sex of plants, preferably prior to planting.

Sex in hemp is genetically determined by a pair of heteromorphic sex chromosomes; females have an XX chromosome pair whereas males have XY. However, environmental conditions (e.g., photoperiod and temperature) and phytohormones can affect sexual phenotype [15,76–79], suggesting other overriding regulatory mechanisms are involved in determining sex in hemp. Monoecious cultivars possess XX sex chromosomes [80], but they produce flower clusters with male flowers at the bottom and females towards the top of each inflorescence [14]. Notably, male flowers occur as the plant transitions from rapid growth to flowering. Stem elongation and fiber development are associated with elevated levels of gibberellins [81]. In hemp, gibberellins are associated with plant masculinity and greater fiber number, length, and diameter [77,82,83]. Thus, a concentration gradient of gibberellin and other hormones may dictate inflorescence sex.

Genetic markers have been developed to differentiate sex in hemp plants [84–86]; however, such a method is not viable for commercial plantings. Recently, quantitative trait loci (QTLs) were identified for sex expression in dioecious and monoecious hemp [87,88]. Cloning of the responsible genes from these QTLs will greatly improve our understanding of genetic control of sex in hemp. Identification of genes present on the sex chromosomes, especially outside the pseudoautosomal recombinant region [86], will be critical for understanding sex-linked traits. Continued development of molecular markers is needed to improve QTL mapping resolution and for marker-assisted selection of desirable traits in breeding programs.

Hemp Molecular Biology

The organic food market is a key player in promoting hemp food and CBD products. As such, widespread public acceptance of transgenic hemp is unlikely. It also remains unknown whether the public will welcome hemp modified using gene-editing techniques, which lack nonplant or plant-pest DNA sequences [89,90]. Thus, many improvements to hemp will probably be accomplished using traditional breeding methods. However; for research purposes, the development of applicable molecular biology techniques is imperative to further study the molecular mechanisms that determine important traits in hemp.

Publication of a draft-quality Cannabis genome and other genetic studies have shed some light into the difference between marijuana and hemp. The Cannabis draft genomeAppendix A[iii] has been compared with draft genome sequences of its closest relative common hop (*Humulus lupulus*; Cannabaceae) as well as more distant species including breadnut (*Artocarpus camansi*; Moraceae) and mulberry (*Morus notabilis*; Moraceae) [91–94]. Recently, low coverage (4–6X) whole-genome sequencing and genotyping-by-sequencing have been performed on 54 (11 hemp and 43 marijuana) and 325 (55 hemp and 213 marijuana) distinct cultivars, respectively [94–97]. However; with only raw data files available, the lack of websites with easy-to-use graphical user interfaces for data analyses limits the utility of these draft-level genome sequences. Transcriptome assemblies are also available (Medicinal Plants Genomes Resource and PhytoMetaSyn databases), but are primarily targeted toward understanding phytocannabinoid metabolism. Comparison of marijuana and hemp indicates that the expression of phytocannabinoid biosynthetic genes is higher in marijuana, suggesting that transcriptional regulation of the pathway may be one factor controlling cannabinoid production [94]. Recently, a transcriptome was generated for hemp bast fibers at different growth stages [98], providing insight into fiber development. The evolution of genetic differences between seed/oil, fiber, and dual-purpose cultivars is less studied. In-depth genetic comparisons of diverse seed/oil, fiber, and phytocannabinoid cultivars are needed to identify the specific genes and mechanisms controlling important yield traits. To attain the full benefit of these and other studies across species, the genome sequence needs to be improved beyond draft quality, and websites with user-friendly graphical user interfaces must be developed.

To characterize hemp gene functions, methods to manipulate gene expression (e.g., via gene knockout or overexpression) are urgently needed. Protocols for developing transformed hairy roots and cell suspension cultures are available [99,100], but the utility of both methods is limited since neither tissue produces seed, fiber, or phytocannabinoids. A whole-plant regeneration protocol has been developed for marijuana [101], suggesting that the development of transgenic hemp plants is feasible. Virus-induced gene silencing methods would also prove useful for studying gene function, but thus far have been unsuccessful [64]. Alternatively, isolation of mutants from chemical mutagenesis screens is possible [32], but extremely difficult due to the anemophilous and dioecious nature of hemp. Currently, exploitation of the natural genetic diversity present within hemp may be the most straightforward way to study gene functions.

Concluding Remarks

Hemp is an unusually diverse crop that can contribute to the seed/oil, fiber, and medicinal product markets. The global market for products derived from hemp is anticipated to double by 2020, largely due to growth in the USA market. The areas of developing seed shattering resistance; increasing seed size; selecting for grain flavors; understanding the microbial populations involved in retting; characterizing and enhancing the properties of hemp useful for biofuel applications; elucidating environmental, hormone, and nutritional impacts on production and accumulation of CBD and other valuable metabolites; establishment of a core hemp germplasm collection; identification of methods to specifically manipulate hemp sex expression as desired; and developing a high quality reference genome with user-friendly interface need further research to improve crop yield to maintain long-term sustainable production and economic viability. Many needed crop improvements can be achieved through traditional plant breeding. However, studies to elucidate the underlying biology of hemp seed, fiber, and metabolite production are lacking (see Outstanding Questions). Immediate establishment of molecular biology techniques is essential to hemp research. Improvements in hemp genomics will advance our understanding of key agronomic traits. While many scientific advances are needed to revitalize hemp production, we have illustrated target areas which we have identified as top research priorities.

Acknowledgements

We would like to thank Rich Mundell and Dr. David Williams for their invaluable insights into hemp research and for providing us with hemp seed for research. We also thank Doris Hamilton and the Kentucky Department of Agriculture for providing production statistics. We are grateful for Dr. David Zaitlin for his critical comments of the manuscript. This work is supported partially by the Harold R. Burton Endowed Professorship to L.Y. and by the National Science Foundation under Cooperative Agreement no. 1355438.

Outstanding Questions

The genetics of hemp grain yield traits remains poorly understood. What mechanisms control yield and quality traits (shattering resistance, sex determination, flavor, and oil composition)? To what extent do shattering, immature seeds, and nonoptimal sex impact grain yield? Answers to these questions are needed to maximize grain production. Both traditional and molecular genetic studies will be instrumental to better understand these processes.

Fiber production is hindered by inconsistent product quality, primarily due to problems with the retting process. How can consistent high-quality retting be attained? While recent efforts have started identifying microorganisms involved with retting, more work is needed to fully understand this key process. Identification of microbe species responsible will help guide development of methods and products, which should improve retting consistency and, in turn, maximize fiber yield and quality.

While the phytocannabinoid biosynthetic pathway is mostly known, the regulation and other mechanisms controlling metabolite quantity remain ambiguous. Specifically, phytocannabinoid yield is known to vary considerably between hemp cultivars and within different environments. Despite this, little is known about environmental impacts on hemp metabolism. How does the environment influence phytocannabinoid levels? What role do hemp specialized metabolites contribute to stress tolerance? Studies are needed to identify regulatory factors controlling phytocannabinoid production, particularly those connected with responses to stress.

Progress has been made to understand the differences between hemp and marijuana. However, much less remains known about the genetic differences between fiber and seed/oil hemp cultivars. What genetic changes separate fiber, seed/oil, or dual purpose hemp cultivars? Identification of such changes will aid breeding efforts selecting hemp for specific purposes.

Resources

[i]clinicaltrials.gov

[ii]omafra.gov.on.ca/english/crops/facts/00-067.htm

[iii]http://genome.ccbr.utoronto.ca/cgi-bin/hgGateway

[iv]votehemp.com/legislation.html

Supplemental Information

Supplemental information associated with this article can be found online at http://dx.doi.org/10.1016/j.tplants.2017.08.004.

References

1. Li, H.-L. (1974) An archaeological and historical account of cannabis in China. *Econ. Bot.* 28, 437–448
2. Pain, S. (2015) A potted history. *Nature* 525, S10–S11
3. Hill, K.P. (2015) Medical marijuana for treatment of chronic pain and other medical and psychiatric problems: a clinical review. *J. Am. Med. Assoc.* 313, 2474–2483
4. National Academies of Sciences, Engineering, and Medicine (2017) *The Health Effects of Cannabis and Cannabinoids: The Current State of Evidence and Recommendations for Research,* National Academies Press
5. Grotenhermen, F. and Müller-Vahl, K. (2016) Medicinal uses of marijuana and cannabinoids. *CRC Crit. Rev. Plant Sci.* 35, 378–405
6. Johnson, R. (2014) *Hemp as an Agricultural Commodity,* Congressional Research Service
7. Callaway, J.C. (2004) Hempseed as a nutritional resource: an overview. *Euphytica* 140, 65–72
8. Galasso, I. *et al.* (2016) Variability in seed traits in a collection of *Cannabis sativa* L. genotypes. *Front. Plant Sci.* 7, 688
9. Simopoulos, A.P. (2002) The importance of the ratio of ω-6/ω-3 essential fatty acids. *Biomed. Pharmacother.* 56, 365–379
10. Karus, M. and Vogt, D. (2004) European hemp industry: cultivation, processing and product lines. *Euphytica* 140, 7–12
11. Carus, M. *et al.* (2013) *The European Hemp Industry: Cultivation, Processing and Applications for Fibres, Shivs and Seeds,* European Industrial Hemp Association (EIHA)
12. Mehmedic, Z. *et al.* (2010) Potency trends of Δ9-THC and other cannabinoids in confiscated cannabis preparations from 1993 to 2008. *J. Forensic Sci.* 55, 1209–1217
13. Andre, C.M. *et al.* (2016) *Cannabis sativa*: the plant of the thousand and one molecules. *Front. Plant Sci.* 7, 19
14. Small, E. (2015) Evolution and classification of *Cannabis sativa* (marijuana, hemp) in relation to human utilization. *Bot. Rev.* 81, 189–294
15. Hall, J. *et al.* (2012) Review of flowering control in industrial hemp. *J. Nat. Fibers* 9, 23–36
16. Russo, E.B. (2007) History of cannabis and its preparations in saga, science, and sobriquet. *Chem. Biodivers.* 4, 1614–1648
17. McPartland, J. (1996) A review of cannabis diseases. *J. Int. Hemp Assoc.* 3, 19–23
18. Amaducci, S. *et al.* (2015) Key cultivation techniques for hemp in Europe and China. *Ind. Crops Prod.* 68, 2–16
19. Salentijn, E.M.J. *et al.* (2015) New developments in fiber hemp (*Cannabis sativa* L.) breeding. *Ind. Crops Prod.* 68, 32–41
20. Cherney, J. and Small, E. (2016) Industrial hemp in North America: production, politics and potential. *Agronomy* 6, 58
21. Fike, J. (2017) Industrial hemp: renewed opportunities for an ancient crop. *CRC Crit. Rev. Plant Sci.* 35, 406–424
22. Gray, D.J. *et al.* (2016) Current and future needs and applications for cannabis. *CRC Crit. Rev. Plant Sci.* 35, 425–426
23. Clarke, R.C. and Merlin, M.D. (2016) Cannabis domestication, breeding history, present-day genetic diversity, and future prospects. *CRC Crit. Rev. Plant Sci.* 35, 293–327
24. Montford, S. and Small, E. (1999) A comparison of the biodiversity friendliness of crops with special reference to hemp (*Cannabis sativa* L.). *J. Int. Hemp. Assoc.* 6, 53–63
25. Fortenbery, T.R. and Bennett, M. (2004) Opportunities for commercial hemp production. *Rev. Agr. Econ.* 26, 97–117
26. Larson, G. *et al.* (2014) Current perspectives and the future of domestication studies. *Proc. Natl. Acad. Sci. U. S. A.* 111, 6139–6146
27. Meyer, R.S. *et al.* (2012) Patterns and processes in crop domestication: an historical review and quantitative analysis of 203 global food crops. *New Phytol.* 196, 29–48
28. Hillig, K. (2005) Genetic evidence for speciation in Cannabis (Cannabaceae). *Genet. Resour. Crop Evol.* 52, 161–180
29. Callaway, J.C. (2004) Hemp seed production in Finland. *J. Ind. Hemp* 9, 97–103
30. Slaski, J. *et al.* (2015) *Industrial Hemp Enterprise,* Alberta Department of Agriculture and Forestry
31. Williams, D. and Mundell, R. (2016) *Agronomic Recommendations for Industrial Hemp Production in Kentucky,* University of Kentucky
32. Bielecka, M. *et al.* (2014) Targeted mutation of Δ12 and Δ15 desaturase genes in hemp produce major alterations in seed fatty acid composition including a high oleic hemp oil. *Plant Biotechnol. J.* 12, 613–623
33. Liu, M. *et al.* (2015) Effect of harvest time and field retting duration on the chemical composition, morphology and mechanical properties of hemp fibers. *Ind. Crops Prod.* 69, 29–39
34. Paridah, M.T. *et al.* (2011) Retting process of some bast plant fibres and its effect on fibre quality: a review. *BioResources* 6, 5260–5281
35. Di Candilo, M. *et al.* (2000) Preliminary results of tests facing with the controlled retting of hemp. *Ind. Crops Prod.* 11, 197–203
36. Jankauskienė, Z. *et al.* (2015) Chemical composition and physical properties of dew- and water-retted hemp fibers. *Ind. Crops Prod.* 75 (Part B), 206–211
37. Müssig, J. and Martens, R. (2003) Quality aspects in hemp fibre production – influence of cultivation, harvesting and retting. *J. Ind. Hemp* 8, 11–32
38. Bennett, S.J. *et al.* (2006) Effect of variety, seed rate and time of cutting on fibre yield of dew-retted hemp. *Ind. Crops Prod.* 24, 79–86
39. Mediavilla, V. *et al.* (2001) Influence of the growth stage of industrial hemp on the yield formation in relation to certain fibre quality traits. *Ind. Crops Prod.* 13, 49–56
40. Ribeiro, A. *et al.* (2015) Microbial diversity observed during hemp retting. *Appl. Microbiol. Biotechnol.* 99, 4471–4484
41. Di Candilo, M. *et al.* (2010) Effects of selected pectinolytic bacterial strains on water-retting of hemp and fibre properties. *J. Appl. Microbiol.* 108, 194–203
42. Tamburini, E. *et al.* (2003) Characterization of bacterial pectinolytic strains involved in the water retting process. *Environ. Microbiol.* 5, 730–736
43. Liu, M. *et al.* (2017) Comparison of traditional field retting and *Phlebia radiata* Cel 26 retting of hemp fibres for fibre-reinforced composites. *AMB Express* 7, 58
44. Cole, C. and Zurbo, B. (2008) *Industrial Hemp – a New Crop for NSW,* Department of Primary Industries (New South Wales)
45. Amaducci, S. *et al.* (2002) Plant population effects on fibre hemp morphology and production. *J. Ind. Hemp* 7, 33–60

46. Lisson, S.N. and Mendham, N.J. (2000) Cultivar, sowing date and plant density studies of fibre hemp (*Cannabis sativa* L.) in Tasmania. *Aust. J. Exp. Agric.* 40, 975–986
47. Augustinović, Z. *et al.* (2012) Stem yield of hemp cultivar Kompolti in relation to plant density and nitrogen fertilization. *Sjemenarstvo* 29, 53–63 (in Croatian)
48. Thomsen, A.B. *et al.* (2005) *Hemp Raw Materials: The Effect of Cultivar, Growth Conditions and Pretreatment on the Chemical Composition of the Fibres,* Risø National Laboratory
49. Burczyk, H. *et al.* (2005) Trends and methods in hemp breeding in Poland. *J. Nat. Fibers* 2, 25–33
50. Struik, P.C. *et al.* (2000) Agronomy of fibre hemp (*Cannabis sativa* L.) in Europe. *Ind. Crops Prod.* 11, 107–118
51. Tolera, A. *et al.* (1998) The effect of stage of maturity on yield and quality of maize grain and stover. *Anim. Feed Sci. Technol.* 75, 157–168
52. Haghighi Mood, S. *et al.* (2013) Lignocellulosic biomass to bioethanol: a comprehensive review with a focus on pretreatment. *Renew. Sustain. Energy Rev.* 27, 77–93
53. Lewandowski, I. *et al.* (2003) The development and current status of perennial rhizomatous grasses as energy crops in the US and Europe. *Biomass Bioenergy* 25, 335–361
54. Keshwani, D.R. and Cheng, J.J. (2009) Switchgrass for bioethanol and other value-added applications: a review. *Bioresour. Technol.* 100, 1515–1523
55. Hodgson, E. *et al.* (2011) Variation in *Miscanthus* chemical composition and implications for conversion by pyrolysis and thermo-chemical bio-refining for fuels and chemicals. *Bioresour. Technol.* 102, 3411–3418
56. Sannigrahi, P. *et al.* (2010) Poplar as a feedstock for biofuels: a review of compositional characteristics. *Biofuel. Bioprod. Biorefin.* 4, 209–226
57. Szczukowski, S. *et al.* (2002) Productivity and chemical composition of wood tissues of short rotation willow coppice cultivated on arable land. *Rostl. Vyroba* 48, 413–417
58. Labrecque, M. and Teodorescu, T.I. (2005) Field performance and biomass production of 12 willow and poplar clones in short-rotation coppice in southern Quebec (Canada). *Biomass Bioenergy* 29, 1–9
59. Prade, T. *et al.* (2012) Energy balances for biogas and solid biofuel production from industrial hemp. *Biomass Bioenergy* 40, 36–52
60. Finnan, J. and Styles, D. (2013) Hemp: a more sustainable annual energy crop for climate and energy policy. *Energy Policy* 58, 152–162
61. Izzo, A.A. *et al.* (2009) Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. *Trends Pharmacol. Sci.* 30, 515–527
62. Booth, J.K. *et al.* (2017) Terpene synthases from *Cannabis sativa*. *PLoS One* 12, e0173911
63. Gagne, S.J. *et al.* (2012) Identification of olivetolic acid cyclase from *Cannabis sativa* reveals a unique catalytic route to plant polyketides. *Proc. Natl. Acad. Sci. U. S. A.* 109, 12811–12816
64. Stout, J.M. *et al.* (2012) The hexanoyl-CoA precursor for cannabinoid biosynthesis is formed by an acyl-activating enzyme in *Cannabis sativa* trichomes. *Plant J.* 71, 353–365
65. Mansouri, H. *et al.* (2013) Ethephon application stimulates cannabinoids and plastidic terpenoids production in *Cannabis sativa* at flowering stage. *Ind. Crops Prod.* 46, 269–273
66. Mansouri, H. *et al.* (2009) Effects of ABA on primary terpenoids and Δ9-tetrahydrocannabinol in *Cannabis sativa* L. at flowering stage. *Plant Growth Regul.* 58, 269–277
67. Mansouri, H. *et al.* (2009) Effects of gibberellic acid on primary terpenoids and Δ9-tetrahydrocannabinol in *Cannabis sativa* at flowering stage. *J. Integr. Plant Biol.* 51, 553–561
68. Happyana, N. *et al.* (2013) Analysis of cannabinoids in laser-microdissected trichomes of medicinal *Cannabis sativa* using LCMS and cryogenic NMR. *Phytochemistry* 87, 51–59
69. Sirikantaramas, S. *et al.* (2005) Tetrahydrocannabinolic acid synthase, the enzyme controlling marijuana psychoactivity, is secreted into the storage cavity of the glandular trichomes. *Plant Cell Physiol.* 46, 1578–1582
70. Small, E. and Naraine, S.G.U. (2016) Size matters: evolution of large drug-secreting resin glands in elite pharmaceutical strains of *Cannabis sativa* (marijuana). *Genet. Resour. Crop Evol.* 63, 349–359
71. Bergman, R. (2014) *I Love Growing Marijuana, Marijuana Plant Care.* Available from http://www.ilovegrowingmarijuana.com/download-free-e-book-marijuana-plant-care/
72. Meier, C. and Mediavilla, V. (1998) Factors influencing the yield and the quality of hemp (*Cannabis sativa* L.) essential oil. *J. Int. Hemp Assoc.* 5, 16–20
73. Welling, M.T. *et al.* (2016) A belated green revolution for cannabis: virtual genetic resources to fast-track cultivar development. *Front. Plant Sci.* 7, 1113
74. Cariñanos, P. *et al.* (2003) Analysis of the particles transported with dust-clouds reaching Cordoba, southwestern Spain. *Arch. Environ. Contam. Toxicol.* 46, 141–146
75. Cabezudo, B. *et al.* (1997) Atmospheric transportation of marihuana pollen from North Africa to the southwest of Europe. *Atmos. Environ.* 31, 3323–3328
76. Mohan Ram, H.Y. and Sett, R. (1994) Reversal of ethephon-induced feminization in male plants of *Cannabis sativa* by ethylene antagonists. *Zeitschrift für Pflanzenphysiologie* 107, 85–89
77. Galoch, E. (1978) The hormonal control of sex differentiation in dioecious plants of hemp (*Cannabis sativa*): the influence of plant growth regulators on sex expression in male and female plants. *Acta Soc. Bot. Pol.* 47, 153–162
78. Mohan Ram, H.Y. and Sett, R. (1982) Induction of fertile male flowers in genetically female *Cannabis sativa* plants by silver nitrate and silver thiosulphate anionic complex. *Theor. Appl. Genet.* 62, 369–375
79. Nelson, C.H. (1944) Growth responses of hemp to differential soil and air temperatures. *Plant Physiol.* 19, 294–309
80. Faux, A.-M. *et al.* (2014) Sex chromosomes and quantitative sex expression in monoecious hemp (*Cannabis sativa* L.). *Euphytica* 196, 183–197
81. Eriksson, M.E. *et al.* (2000) Increased gibberellin biosynthesis in transgenic trees promotes growth, biomass production and xylem fiber length. *Nat. Biotech.* 18, 784–788
82. Ram, H.Y.M. and Jaiswal, V.S. (1972) Induction of male flowers on female plants of *Cannabis sativa* by gibberellins and its inhibition by abscisic acid. *Planta* 105, 263–266
83. Atal, C.K. (1961) Effect of gibberellin on the fibers of hemp. *Econ. Bot.* 15, 133–139
84. Mandolino, G. *et al.* (1999) Identification of DNA markers linked to the male sex in dioecious hemp (*Cannabis sativa* L.). *Theor. Appl. Genet.* 98, 86–92
85. Törjék, O. *et al.* (2002) Novel male-specific molecular markers (MADC5, MADC6) in hemp. *Euphytica* 127, 209–218
86. Peil, A. *et al.* (2003) Sex-linked AFLP Markers indicate a pseudoautosomal region in hemp (*Cannabis sativa* L.). *Theor. Appl. Genet.* 107, 102–109
87. Faux, A.-M. *et al.* (2016) Identification of QTLs for sex expression in dioecious and monoecious hemp (*Cannabis sativa* L.). *Euphytica* 209, 357–376
88. Faux, A.-M. and Bertin, P. (2014) Modelling approach for the quantitative variation of sex expression in monoecious hemp (*Cannabis sativa* L.). *Plant Breeding* 133, 782–787
89. Andersen, M.M. *et al.* (2015) Feasibility of new breeding techniques for organic farming. *Trends Plant Sci.* 20, 426–434
90. Wolt, J.D. *et al.* (2016) The regulatory status of genome-edited crops. *Plant Biotechnol. J.* 14, 510–518
91. Gardner, E.M. *et al.* (2016) Low-coverage, whole-genome sequencing of *Artocarpus camansi* (*Moraceae*) for phylogenetic marker development and gene discovery. *Appl. Plant Sci.* 4, apps.1600017
92. Natsume, S. *et al.* (2014) The draft genome of hop (*Humulus lupulus*), an essence for brewing. *Plant Cell Physiol.* 56, 428–441
93. He, N. *et al.* (2013) Draft genome sequence of the mulberry tree *Morus notabilis*. *Nat. Commun.* 4, 2445
94. van Bakel, H. *et al.* (2011) The draft genome and transcriptome of *Cannabis sativa*. *Genome Biol.* 12, R102

95. Vergara, D. *et al.* (2016) Genetic and genomic tools for *Cannabis sativa*. *CRC Crit. Rev. Plant Sci* 35, 364–377

96. Lynch, R.C. *et al.* (2016) Genomic and chemical diversity in cannabis. *CRC Crit. Rev. Plant Sci.* 35, 349–363

97. Sawler, J. *et al.* (2015) The genetic structure of marijuana and hemp. *PLoS One* 10, e0133292

98. Guerriero, G. *et al.* (2017) Transcriptomic profiling of hemp bast fibres at different developmental stages. *Sci. Rep.* 7, 4961

99. Wahby, I. *et al.* (2012) Agrobacterium infection of hemp (*Cannabis sativa* L.): establishment of hairy root cultures. *J. Plant Interact.* 8, 312–320

100. Feeney, M. and Punja, Z.K. (2003) Tissue culture and *Agrobacterium*-mediated transformation of hemp (*Cannabis sativa* L.). *In Vitro Cell. Dev. Biol. Plant* 39, 578–585

101. Lata, H. *et al.* (2010) High frequency plant regeneration from leaf derived callus of high Δ9-tetrahydrocannabinol yielding *Cannabis sativa* L. *Planta Med.* 76, 1629–1633