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Hemp Breeding and the Uses of Photoperiod Manipulation

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Creative Component Project

Hemp Breeding and the Uses of Photoperiod Manipulation

by

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Introduction

Hemp (*Cannabis sativa* subsp. *sativa*) is a versatile plant worth more than 300 million dollars a year and creates more than 25,000 different kinds of products (Johnson, 2011). It provides not only fiber for the textile industry but also seed, and medicinal cannabidiol (CBD). Nearly all parts of the hemp plant can be used. The stalk of the plant can be used for fiber products. Seed is used for food products and planting, and the flowers of the plant are used for extracting CBD oil.

This versatility makes hemp an interesting plant to work with. Breeding hemp can be very diverse because it can be bred and developed for any of these uses. Plants developed specifically for flower production and fiber are visually very different. Plants bred for seed have different breeding objectives than those developed for flower production.

Perhaps the most interesting aspect of hemp's development is its ability to regulate growth by responding to the duration of light it receives daily. Among the effects of photoperiod manipulation is the ability to change the sex of plants, induce flowering, and rejuvenate flowering plants. Manipulations such as these could be very useful in a breeding program and will be covered in this paper. This paper will also include sections on the botanical nature of hemp, cultural methods, genetics and breeding, and special techniques.

The objective of this creative component is to develop an educational paper covering hemp breeding and the uses of photoperiod manipulation. This project is needed because there is limited research done on the effects of changing the photoperiod in cannabis. In fact, papers on Cannabis in general are not plentiful due to the prior legality of hemp. This paper will provide information that current and prospective hemp breeders will find relevant.

Botany of Hemp

Phylogeny

Hemp is from the family of *Cannabaceae* and the genus of *Cannabis* (McPartland, 2018). Speciation of *Cannabis* is often debated by scientists. They argue whether *Cannabis* has only one species or if it is a multi-species genus (Chandra et al., 2017a). Some taxonomists consider *C. indica* and *C. sativa* to be distinct species and not subspecies of *Cannabis sativa* (Clarke and Merlin, 2016). Others believe that *C. indica* and *C. sativa* are one species. Part of this confusion comes from the fact that all types of cannabis interbreed freely. (McPartland, 2018).

The USDA database and Integrated Taxonomic Information system (ITIS) classify Cannabis as a single species *C. sativa L.* Hemp breeders often include the sub-species *indica* and *sativa* (Green, 2017). The idea that *Cannabis* is a single species comes from Linnaeus. He described the single species *C. sativa* (Linnaeus, 1753 as cited in McPartland, 2017) Lamarck believed that there were two species *C. sativa* and *C. indica* (Lamarck, 1785 as cited in McPartland, 2017) Small and Cronquist (1976) agreed that there was only one species *C. sativa* with several sub-species.

Following the subspecies theory hemp is considered *C. sativa* subsp. *sativa*. Marijuana is considered. *C sativa* subsp. *indica* (McPartland, 2018). Schultes (1974) identified the morphology of *C. sativa* and *C. indica* believing them to be separate species. He also added a third species *C. ruderalis* (Schultes, 1974 as cited in Green, 2017). *C. sativa* subsp. *sativa* is a taller thin stalked plant with narrow leaves, high cannabidiol (CBD) and low Tetrahydrocannabinol (THC) content. *C. sativa* subsp. *indica* is a shorter plant with a woody stem, broad leaves, and a high THC content. Both *C. sativa* and *C. indica* have a photoperiod-sensitive flowering mechanism (McPartland, 2018).

The sub-species *C. sativa* and *C. indica* have a variety under each of them. *C. sativa* subsp. *sativa* var. *spontanea* is a high cannabidiol plant with a short stature and lack of photosensitivity. It flowers automatically at a certain age regardless of day length. This is referred to as "autoflowering" or day neutral. *C. sativa* subsp. *indica* var. *kafiristanica* is a wild type with high levels of tetrahydrocannabinol (McPartland, 2018).

Small (2015) also suggested new designations in agreement with a classification system called the International Code of Nomenclature for Cultivated Plants (ICNCP) that allows groups of cultivars to be recognized. Several new species and subspecies were identified by the leaf structure and THC content. The four types are narrow leaf hemp (C. *sativa* subsp. *sativa*), broad leaf hemp (C. *indica* subsp. *chinensis*), Narrow leaf drug type (C. *indica* subsp. *indica)*, and broad leaf drug (C. *indica* subsp. *afghanica).* Sinsemilla cultivars were presented as hybrids of the narrow and broad leaf types (Small, 2015).

Hops (*Humulus lupulus*) is hemp's closest relative from the family *Cannabaceae* and genus *Humulus* is thought to have diverged around 208 million years ago from the genus *Cannabis*. They share three phenotypic characteristics pollen grains with three furrows, overlapping sepals, and an attached calyx (McPartland, 2018). A second relative hackberry (*Celtis occidentalis)* is also in the *Cannabaceae* family (Green, 2017).

Structure and flowering

Plant structure

Hemp is a dioecious wind-pollinated plant (Clarke and Merlin, 2016). Although, monoecious varieties do exist they are rare in nature. Monoecious plants are a product of domestication (Small, 2017). The morphology of hemp is determined in part by environment and cultural methods. Fiber plants are between 8 and 12 feet tall and plants grown for flowers are usually less than four feet tall (Bessey, 1933). Plants grown for fiber are planted densely and have very few branches. Conversely seed plants are multi-branched and spaced further apart (Britannica, 2019).

Hemp has a long taproot that can penetrate the soil up to a foot deep. From the taproot the plant produces fibrous lateral roots. The deep roots can absorb nutrients that were left behind from other crops. The roots also have the advantage of absorbing water deep in the soil during drought (Canadian Hemp Trade Alliance, 2020).

Hemp has an erect woody stem (Chandra et al., 2017a). The stem of the plant has a hollow center. Inside the stem is the bark that is made up of parenchyma cells and bast fibers. Bast is the primary fiber used in fiber production and is made up of phloem fibers. The fibers are bundled in groups of 10-40 and separated by retting (Grassi and McPartland, 2017). The bast is located just outside of the vascular cambium. Bast fibers are made up of cellulose, pectin and lignin (Salentijn et al., 2015).

Hemp has an opposite phyllotaxy (Schaffner, 1921). The leaves of the hemp plant are compound with a palmate shape (Britannica, 2019). The first true leaves are tri-lobed (Green, 2017). The mature leaves of the plant have a palmate venation and between three to nine leaflets (Raman et. al, 2017). The leaflets are lanceolate shaped (Chandra et al., 2017a). Fan leaves are large outstretched leaves that are near the bottom of the plant which are used to increase photosynthesis (Green, 2017).

Reproductive structures

Male and female plants are morphologically different in the sexual stage. When flowering initiation begins the plant changes its focus from vegetative growth and focuses on the production of flowers and seeds (Salentijn et al, 2019). At the onset of the reproductive phase the phyllotaxy will change from an opposite to an alternate pattern (Salentijn et al, 2019). When the plant is ready to transition into reproductive phase it will begin to pre-flower. Pre-flowering is the beginning of calyx development and is not light dependent. (Green, 2017 Figure 1A, D).

When hemp reaches the reproductive stage the male and female plants begin to differentiate (Clarke and Merlin, 2016). The male plant becomes spindly and taller than the more robust female plant. It also has fewer leaves than the female plant with more visible flowers (Bessey, 1933), a smaller root system, and a shorter life span (Schaffner, 1921). The males die soon after flowering (Bessey, 1933). The flower consists of five sepals that are oblong in shape with five fiber-like stamens and oblong hanging anthers (McPartland and Guy, 2017 Figure 1B).

The females give off a strong odor when flowering (Bessey, 1933). The female flower has a single sepal that tapers to a point. The pistil has a minute ovary, sharply pointed and divided style, and an acute stigma (McPartland and Guy, 2017). The female plant has tightly clustered flowers with tiny leaflets. Clusters of flowers are known in hemp as "buds" (Small, 2017 Figure 1C).

Female flowers produce a sticky resin to catch pollen (Clarke and Merlin, 2017). The resin is high in cannabinoids (Grassi and McPartland, 2017). Resin has several other purposes including anti-fungal properties, insect resistance, and protection from animals (Green, 2017). The developing seeds are surrounded by a protective bract and covered in thousands of trichomes (Clarke and Merlin, 2016), the producers of cannabinoids.

Figure 1 **Male and female cannabis pre-flowers and flowers**
A) male Pre-flower B) male flower C) female flower D) female pre-flower

Flowering Initiation

For the plant to reach the reproductive stage flowering must be initiated by the correct photoperiod. Hemp is a short-day plant and long photoperiods will keep hemp plants in a vegetative state. Hemp will not flower when introduced to photoperiods over 16 hours a day. Flowering is initiated in light periods under 14 hours a day (Borthwick and Scully, 1954). In order to flower hemp needs at least 10-12 hours of darkness (Salentijn et al. 2019).

In a breeding program supplemental light can be used to keep plants from flowering when days start to shorten. After the light is removed the plants will quickly start flowering in as little as two weeks (Borthwick and Scully, 1954). Male and female plants do not flower simultaneously. The male flowers before the female and dies after flowering (Salentijn et al. 2019). The photoperiod can be manipulated so that both go into flower at the same time. Using photoperiod treatment flowering can also be induced in varieties that flower at different times so that they can be crossed together. Using shortened photoperiods in the greenhouse several generations of plants can be produced in a year (Borthwick and Scully, 1954).

Domestication

Hemp is thought to have originated in Eurasia (Clarke and Merlin, 2016). According to fossil records *Cannabis* originated in the Tibetan plateau. Fossil pollen evidence also points to hemp being in Europe between 1.2 and 1.8 million years ago (McPartland, 2018). Domestication was thought to first have happened around 6500 years ago in Mongolia and hemp is extensively bred in China (Green, 2017).

Hemp is one of the oldest sources for food and textiles. Hemp trade originated in China and was spread throughout the world and it is found in the Middle East, North America, South America, Europe, and the tropics (Chandra et al., 2017a). It was likely used by ancient people and was one of the first cultivated plants. Factors that contributed to its widespread distribution was its weedy nature, need for fertile soil, and multiple uses (Clarke and Merlin, 2016).

Cannabis has been critical to the advancement of civilization. It was used medicinally in the ancient world. It also has a long history of being used by humans. It was found in clay pots from 10,000 BCE, harvested in China as long as 8500 years ago. It is thought to be deliberately cultivated and transported at least for 6000 years. Hemp seeds were considered a grain in China and were found in 4500 year old tombs (Small, 2017). Hemp was used for fiber as early as 2800 BCE and spread from Europe to Chile, and to North America by the 1600's (Britannica, 2019).

Hemp exists both as a cultivated plant and as a feral population of cultivated plants that are growing wild (Pollio, 2016). Wild populations are likely feral populations that at some point have been crossed with domesticated hemp (Small, 2017). Traditionally local farmers domesticated wild plants and made selections, creating landraces. Landraces are plants selected and bred by farmers for the local area and are not part of a breeding program (Green, 2017).

Hemp grows in temperate climates in well-drained soil. It grows mostly in the northern hemisphere (Clarke and Merlin, 2016). It can be found growing wild in Mongolia, Tibet, the Himalayas, and India (Green, 2017). Hemp was grown in northern

Asia for fiber and seed and because of the latitude in northern Asia, hemp from that area is early flowering (Small, 2017).

Domestication is the process of fixing traits that are useful to humans (Small, 2017). Cannabis is one of the earliest plants that have been domesticated with a long history of cultivation (Raman et al., 2017). An important product of domestication is non-shattering seeds because in the wild seeds are easily dispersed through shattering. A trait that is not favorable to produce seed crops (Small, 2017) because of the significant loss of yield (Schluttenhofer, 2017).

Through domestication hemp has been selected for traits favorable to the production of fiber, seed, and flower. These include more fiber content and larger seed sizes (Clarke and Merlin, 2016). Increased fiber content is useful in fiber production. Larger seeds are useful in both seed and propagation because it provides more nutrition and better germination. Domesticated hemp also has larger broader leaves which provide an advantage in photosynthesis (Small, 2017).

Economic importance

There are three major economic uses for hemp. It is grown for fiber, seed, or flowers. Hemp used for fiber and seed crops is often referred to as industrial hemp (Small, 2017). The revenue of CBD was up to $25000 per hectare in 2015. Fiber was worth up to $4848 and grain $2841 per hectare. The economic value of hemp is substantial and was predicted to double from the 2015 numbers by 2020. The United States is the biggest importer of seed and fiber and hemp is also grown and sold in 47 other countries (Schluttenhofer, 2017). Registered acres of hemp in the United States has grown from 37,000 in 2017 to over 310,000 acres in 2019 (Sterns, 2019).

Fiber hemp is of minor economic importance (Small, 2017). Both bast and hurd from fiber crops are used for making things like animal bedding, biofuel, and paper products (Schluttenhofer, 2017). In fiber hemp bast fibers are extracted through a process called retting. It is dried and removed from the outside stem. The fibers are also used for cordage, bioplastics, and linen (Britannica, 2019). It is also important in the construction industry for making hempcrete out of hurds and lime (Canadian Hemp Trade Alliance, 2020).

Hemp for seed is currently produced in China, Europe, and Canada. Hemp seeds provide a multipurpose vegetable oil called hempseed oil (Small, 2017) which has many uses including food products meant for animals and humans (Schluttenhofer, 2017). The seed can be eaten by humans, used as bird feed, or oil-based products such as varnish (Britannica, 2019).

CBD, extracted from flowers, is often used medicinally and with less than 0.3% THC it does not cause intoxication. CBD is being investigated to treat ailments such as anxiety, drug dependencies, and schizophrenia. It is designated as a probable treatment for rare diseases such as Fragile X syndrome, and spasms in infants (Schluttenhofer, 2017). It is also used to treat a type of seizure in children called intractable pediatric epilepsy with promising results (Chandra et al., 2017a).

Propagation and cultural practices

Propagation

Hemp can be propagated through both clones and seeds. Cloning creates a uniform and consistent crop. Clones can be taken from selected females and kept in a vegetative state. The main use of cloning is to fix the trait of the parent cultivar. Female and male clones can be crossed together to find the best male/female pairings to meet breeding objectives. This produces both males and females. Female clones can be crossed to clones from the same mother plant to make a self-fertilized generation without losing vigor by self-fertilizing for several generations and restoring vigor by crossing with an inbred cultivar (Clarke and Merlin, 2016).

Clones are cut from vegetative parts of a “mother” plant for clonal propagation. About 12 cm of the stem is cut off the mother plant at a 45-degree angle. It is then dipped in a rooting solution and placed in a rooting cube. The clone is then held under twenty-four-hour lighting at 25℃ in a high humidity environment. The clone stays in this environment for two weeks until it develops roots and is transferred to a pot in the greenhouse or directly into the field (Chandra et al., 2017b).

Greenhouse production

Once planted in the greenhouse plants are grown in a vegetative state for three weeks. To keep them in vegetative state they are placed under artificial lighting for a period of eighteen or twenty-four hours a day. Eighteen-hour schedules conserve electricity but twenty-four-hour schedules promote increased growth. The greenhouse is kept at 25°C and aerated using fans. After three weeks plants are ready to transition to the reproductive cycle and the light cycle is changed to twelve hours light per day. After 10 weeks in the reproductive stage plants are then harvested and hung to dry. Greenhouse production is mainly used for CBD production and after drying the leaves and flowers are stripped from the stalk and the stem discarded (Chandra et al., 2017b).

Field production

Hemp for field production is more commonly sown rather than propagated by clones, for which production costs could be prohibitive (Chandra et al., 2017b). The seeding rate for seed production is 100 to 125 seeds per square meter. For fiber production that rate is increased to 200 to 300 seeds per square meter (Canadian Hemp Trade Alliance, 2020). This number should be reduced if growing a row crop. Rows can be spaced up to 18cm apart and sown at 100 to 200 plants per square meter (Fikes, 2017). Hemp grows best in well-drained soil. Waterlogging leads to chlorosis of the growing points, killing the plant (Canadian Hemp Trade Alliance, 2020).

In Canada, the optimum time to plant hemp is between May 16th and June 10th. If planted early the soil may be too cold. Later plantings may reduce yield and height of plants. Regardless of the seed planting date plants will flower when the photoperiod shortens to less than 16 hours a day (Canadian Hemp Trade Alliance, 2020).

Depending on field conditions and variety it takes hemp 90-120 days to mature (Canadian Hemp Trade Alliance, 2020). Seed and flower crops are mechanically harvested and dried in the field (Chandra et al, 2017b). Seed plants are harvested either when they start to shatter or when 80% of the seeds are mature. Fiber crops are harvested and left on the ground for a process known as retting. Retting is the degradation of pectin by microbes and may be sped up by natural precipitation or a few inches of water. Fiber crops are harvested after the males shed pollen and before seeds set in the females (Canadian Hemp Trade Alliance, 2020).

Genetics

Genetic research

Most research on hemp can be found up until the mid-1900's when it was banned by the Marihuana Tax Act and completely outlawed by the Controlled Substances Act in 1970 (Hemp timeline, 2019). The United States Agricultural Act of 2014 made it legal for some research institutions and state agencies to grow hemp (Johnson, 2011). Hemp wasn't federally legal until the farm bill in 2018. Hemp or "industrial hemp" is classified as being having less than 0.3% THC. This classification extends worldwide but hemp must have less than 0.2% THC in Europe (Salentijn et al., 2015).

The *Cannabis* genome was sequenced and published in 2011. The hemp genome has a diploid number of 20 with 18 autosomes and 2 sex chromosomes. The female genome is 818Mb and the male 843Mb (Bakel et al, 2011). Many roadblocks in sequencing existed including the heterozygous nature of the genome, repetitive DNA, and polymorphisms. Researchers used a combination of genetic mapping, physical mapping, and long read sequencing to overcome these hurdles (Salentijn et al, 2019).

Gene expression and function research and knowledge is limited. Molecular markers have been developed for flowering times in *Arabidopis thaliana* and rice. The identification of genes can be done by comparing similar genes in other crops. This should be used with caution as the genes may not be the same or perform the same function. Markers for flowering time in hemp would be desirable (Salentijn et al, 2019).

Hemp has so far not been successfully modified by genetic modification (Salentijn et al., 2019). While transgenic hemp may not be commercially viable, it may prove useful in understanding molecular mechanisms (Schluttenhofer, 2017).

There are many areas that still need research in hemp. The use of molecular markers is limited because of the high level of heterozygosity of hemp (Salentijn et al., 2019). Research on phytocannabinoids is needed specifically for early steps of the cannabinoid pathways and their regulation. Although some genetic markers and quantitative trait locus have been developed that determine sex expression in hemp more research on sex-linked traits and QTL mapping is important for reaching breeding objectives in photocannabinoid development and regulation (Schluttenhofer, 2017).

Sex determination

Hemp plants have 18 autosomes and two sex chromosomes (Bessey, 1933). Sex is controlled by X and Y chromosomes (Clarke and Merlin, 2016). The female karyotype is XX and the male karyotype is XY (Salentijn et al., 2019). Schaffner (1921) proposed that it would be possible to create a monoecious species under normal conditions, if there was a change in the sex chromosomes. A change in sex chromosomes is not necessary. When monoecious plants are tested, they are genetically XX and because of this it is believed that male determination may not be on the Y chromosome (Salentijn et al., 2019).

According to Truta et al. (2007) male flowers on female plants may be genetically determined by a separate masculinizing gene. This may be the X_m allele. In this theory male plants with male flowers would be XY or X_mY. Male plants with female flowers would be X_m and monoecious plants would be X_mX.

Breeding

Cultivars

Rather than using the term cultivar current hemp breeders often use the term strain. Strains do not meet the necessary requirements found in the ICNCP to be considered a cultivar (McPartland and Guy, 2017). This is due in part to the fact that they are not registered and are without description or standards (Pollio, 2016). Most available strains are also highly unstable. This is especially true of hybrid strains (Green, 2017).

Due to the changes in laws and the given 0.3% THC threshold some varieties have been stabilized and registered as cultivars. In Europe there are currently 51 registered cultivars (Salentijn et al., 2015). The THC threshold has also made it possible for Canadian breeders to register their varieties as cultivars without legal obstacles (Laursen, 2015).

Breeding objectives

Populations for fiber production utilize monoecious, female, and male plants. Stabilized monoecious plants are preferred because they are more uniform than dioecious plants (Salentijn et al., 2019). Male plants have a better fiber quality than female plants (Small, 2017). For seed production a few males or monoecious plants are necessary to supplement a mainly female population (Schluttenhofer, 2017). A large number of males in a population will lead to a reduction in seed yield (Salentijn et al., 2019). CBD production requires a solely female population (Schluttenhofer, 2017).

Breeding goals of fiber hemp include having good fiber quality, a low THC content, lodging resistance, and flowering times (Salentijn et al., 2015), high cellulose content, low pectin, and lower lignin cross linkages (Schluttenhofer, 2017). Another goal of fiber breeders is to have cannabinoid-free cultivars by reducing the number of trichomes and resin. Breeders are also working on fast retting varieties to speed up the retting process (Grassi and McPartland, 2017).

Varieties are bred according to the latitudes and growing conditions. Salentijn (2019) proposed regional breeding for reduced photosensitivity in the north and development of late flowering varieties to maximize fiber quality and yield in those areas. This is possible because late flowering increases stem mass. Monoecious cultivars that flower early are bred for dual production purposes (Salentijn et al., 2019).

Breeding for fiber quality is needed because of fiber variability (Salentijn et al., 2015). Breeding for shorter and less lignified secondary bast fibers would improve the quality of fiber hemp (Salentijn et al, 2019). Fiber crops are bred to have more bast fiber than the inner woody fiber, with limited hurds, and tall unbranched stems. Plants with less of a woody center are more susceptible to lodging but the practice of planting in high densities reduces the likelihood (Small, 2017).

There are not many cultivars bred specifically for producing seed. Most seed crops are from fiber plants that are harvested both for fiber and seed (Grassi and

McPartland, 2017). Breeding objectives for seed include yield, seed quality, flowering time, nutrients for human consumption (Salentijn et al., 2019), larger and non-shattering seeds (Schluttenhofer, 2017).

Breeding for short branches and compact flowers is favorable for mechanical harvest and a reduction in stem production. High-yielding uniform crops with seeds that mature at the same time are also favorable (Small, 2017). Oil contains useful compounds needed in human diets. They contain polyunsaturated fatty acids such as omega 3 and 6 (Grassi and McPartland, 2017). A breeding objective for oil as product is breeding for flavor and increased omega 3 fatty acids. This would expand the use in human food products (Schluttenhofer, 2017).

An objective of breeding for dual-purpose plants is for monoecious varieties. They are favorable because monoecious plants are more uniform and have a higher yield of seeds, but the trait must be maintained (Salentijn et al., 2015). Monoecious lines rarely occur naturally and must be bred and selected for or they will return to the natural dioecious state (Clarke and Merlin, 2016).

The THC to CBD ratio is referred to as the chemotype. It can be measured anytime from seedling to flowering plants (Grassi and McPartland, 2017). Cannabinoid levels are not only controlled by genetic factors. Environmental factors also influence the percentage of cannabinoids that are produced (Grassi and McPartland, 2017).

The economic interests of CBD have led breeders to work on developing cultivars with high CBD but low THC (Grassi and McPartland, 2017). Trichomes are the resin producing gland of the hemp plant and contain the greatest amount of cannabinoids. Cannabis used for cannabinoid production is bred to have a greater number of trichomes and more compact inflorescences (Small, 2017). Terpenoids are an aromatic compound found in the flower's essential oils. They can be bred for different aromas and used in beauty products (Grassi and McPartland, 2017).

Breeding techniques

Unlike breeding for traditional crops such as corn, hemp breeding is limited by the lack of available accessions (Schluttenhofer, 2017). This is due in part to legal restrictions causing cannabis breeders to work in secret with small populations and little genetic diversity. To properly breed cannabis a large population is needed to obtain the traits needed for a successful breeding program (Clarke and Merlin, 2016).

Environmental conditions play a role in the expression of most traits. Hemp grown in the field is often more robust and has a higher biomass than when grown indoors (Chandra et al., 2017a). The different densities at which crops are planted serve to promote the phenotypic attributes needed for each particular use. The dense planting of fiber plants promote a long stalk with few branches. Looser plantings promotes branching and flowering (Clarke and Merlin, 2016).

There are several ways to breed hemp both in the monoecious and dioecious forms. Monoecious varieties can be self-fertilized unlike dioecious hemp which is an obligate crossbred plant. Hemp can be bred using techniques such as mass selection, crossbred, inbred or crossed as hybrids.

Population development

Most of the products besides fiber comes solely from female plants. This makes it difficult to choose traits from male plants because they will ultimately be expressed in the female plant in flower and seed crops. Selection practice is often limited to the selection of female traits. This impacts the males which become shorter with more flowers like females (Clarke and Merlin, 2016). When selecting males for fiber plants it is common to select before pollination and pollinate with the best males (Salentijin et al, 2015). Male plants may be silent carriers of traits such as terpenes and bud formation. The only way to determine the male traits passed on is to grow out and evaluate the offspring (Green, 2005)

Hemp is often open-pollinated and reproduced through half-sibs. In open pollination the male pollinator can not be controlled. Pollination using a single male plant can be controlled one of three ways: through clonal propagation, isolation or by collecting pollen and fertilizing each female. A male can be cloned and used in a field or a greenhouse to produce a full-sib population. Another way is to isolate a group of females in a greenhouse and use fans to open pollinate them with a single male. The third way is to use a paper bag tied to a male flower to collect pollen and use a brush to artificially pollinate a female bud (Green, 2005).

Mass selection

Traditionally hemp breeding was done by mass selection and recurrent selection (Grassi and McPartland, 2017). Mass selection is used to create homogenous lines. This is useful for breeding simple traits that are highly heritable (Clarke and Merlin, 2016) such as bast fiber quality. However, mass selection is slow to improve fiber quality because pollination cannot be controlled (Salentijn et al., 2015). In mass selection seeds are taken from selected females, and males are chosen before shedding pollen. The field is then fertilized through open pollination (Clarke and Merlin, 2016). Seeds can be selected in one of two ways: 1) The entire field can be harvested, and seeds chosen from the bulk or 2) seed is harvested from selected plants. With recurrent mass selection the process is repeated until a homogenous population is achieved.

Controlled hybridization

An alternative to open pollination is controlled hybridization. With controlled hybridization a female plant is crossed with a single favorable male rather than being open-pollinated. The offspring are then open-pollinated to form the F_2 generation. The F_2 generation is evaluated and chosen individuals are advanced in either full or half-sibling selection (Clarke and Merlin, 2016).

Inbreeding

Monoecious cultivars or females with artificially forced male flowers through sex reversal techniques can be inbred. Inbreeding is achieved by multiple generations of self-fertilization which can cause inbreeding depression. This is overcome by crossing the new inbred to a genetically different inbred line forming a new F_1 generation (Clarke and Merlin, 2016).

Hybrids

Hybrid populations in hemp are commonly done on a small scale for sinsemilla cultivars. Female plants produce a higher percentage of cannabinoids such as CBD with sinsemilla types producing the highest amounts (Chandra et al., 2017a). In hybridization as in controlled hybridization two strains are crossed together to produce a F_1 with traits from both parents. Hybridization is usually done with two inbred strains to form an F_1 with the advantage of heterosis. The F_2 generation will have a loss of vigor and high levels of variation because of presence of recessive alleles (Clarke, 1981). Cloning can be useful in hybridization. For example, heterosis can be maintained by cloning the F_1 generation and or the parents can be maintained as clones to reproduce the hybrids. If breeding is continued, the next generation can be formed in several different ways: the F_1 can be crossed to a full sibling resulting in a heterozygous F_2 population that can be refined using mass selection. It can also be crossed to one of the original varieties increasing homozygosity. The F_2 can then be backcrossed and then used in mass selection. The third way would be to an unrelated parent (Clarke and Merlin, 2016).

Special Techniques

Feminization

McPhee (1924) discovered that seed whose pollen source was an induced male flower on a female hemp plant produced only female offspring (McPhee, 1924 as cited in Borthwick and Scully, 1954). The experiment was repeated by Borthwick and Scully (1954) who experimented with self-fertilized inter-sex females and confirmed McPhee's results.

When creating feminized seed male flowers are induced on a female plant in a process known as sex reversal (Figure 2). Schaffner (1921) used the term sex reversal to indicate the presence of any male flowers on a female plant (Borthwick and Scully, 1954). Sex reversal techniques are used to produce feminized seed which creates an all-female population. It can be useful in hemp plants because it can be used directly in a breeding program by transforming female plants so they can be used as pollinators. An all-female population is beneficial to prevent contamination from male pollen, causing seeding. It also acts as an agent of male sterility, reducing labor in hybrid breeding (Salentijn et al., 2015).

Figure 2 **Male cannabis flowers on a female inflorescence**

One way to cause sex reversal in a female plant is through a silver spray. Lubell and Brand (2018) did an experiment with silver thiosulfate (STS) on female hemp plants. STS promotes synthesis of gibberellic acid which produces male flowers (Salentijn et al., 2015). In their experiment they used three concentrations of STS: 0,0.3 and 3mM. They sprayed three sets of plants three times at a week’s interval.

They found that those receiving 0 STS had normal female flowers. 0.3mM produced flowers with both male and female flowers and 3mM produced completely male flowers. They did note however that that the male flowers produced had a reduced amount of pollen (Lubell and Brand, 2018). Ram and Sett (1982) noted that the leaves sprayed with STS had severe necrosis and stunted growth.

The second way to cause sex reversal is to use photoperiod manipulation. Schaffner did several experiments on sex reversal in hemp. In his experiments he found that there were several intermediate forms when sex was reversed (Schaffner, 1921). Sex reversal also occurs in male plants, but it is less common than in female plants. This may be because the males die soon after flowering and the females may live for months after (Schaffner, 1921). He also found that male plants showing some female expression lived longer than those that expressed strictly male features (Schaffner, 1918).

Schaffner found that low light winter conditions caused male flowers to grow on female plants. Female plants grown in the spring under normal conditions did not produce male flowers. Some of the plants produced had a mix of male and female parts but some showed a complete reversal of sex (Schaffner, 1921). He speculated that sex reversal in hemp is not due to any change in the sex chromosomes but is rather caused by abnormal environments (Schaffner, 1921). In this case sex reversal was caused by light deprivation (Schaffer 1918).

His first set of winter experiments were done in a heated greenhouse. The seed used in each winter planting were the offspring of the plants grown the summer before. The parent plants showed no signs of sex reversal, ruling out genetic factors (Schaffner, 1923).

The plants in the summer garden developed normally and there was no degree of sex reversal found. Of the plants that survived the winter plantings an average of 75% of them showed some degree of sex reversal (Schaffner, 1921).

Plantings were done for two years from 1920 to 1921Schaffner measured sex reversal based on the number of plants showing any sex reversal. The rates of sex reversal ranged from 0% in the long-day summer months from May to August to 93% in the short-day winter from late August to April.

In a second set of experiments 21 successive planting were made between July 1929 and May 1930. Successive planting throughout the year showed that most sex reversals occured during the winter months (Figure 3). Schaffner found that the crops planted between May 15th and July 15th showed no sex reversal at all. Those planted from November 1st to 15th showed a 100% rate of reversal (Schaffner, 1931).

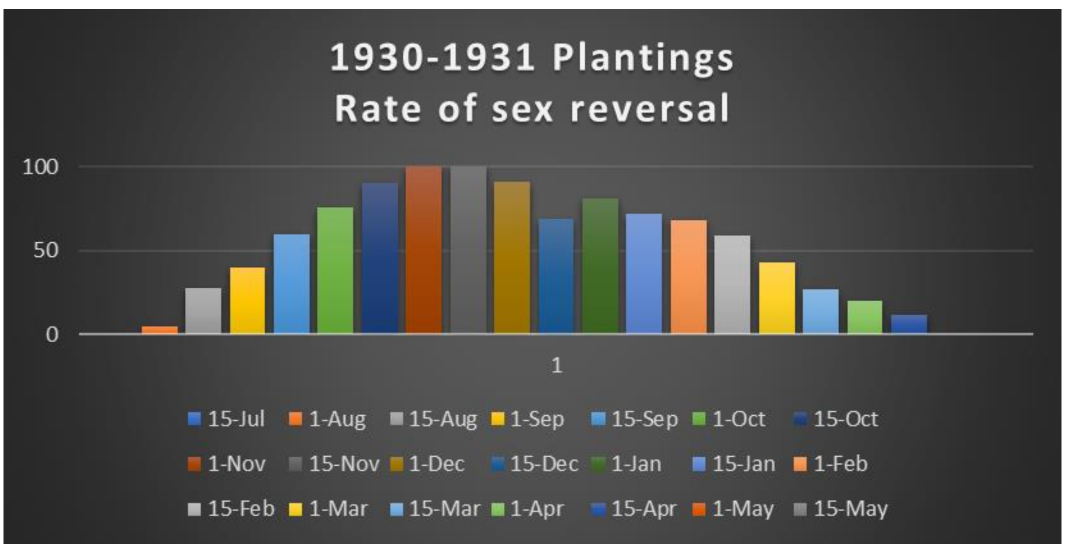

Figure 3 **Rates of sex reversal from Schaffner's 1930's experiments using photoperiod manipulation in hemp**. Schaffner, 1931

McPhee (1925) did similar experiments, pollinating a female plant with male flowers with its own pollen. Out of the 161 seeds produced only three were male. He believed this to be from cross-contamination from a male. He repeated the experiment from the female progeny and obtained only female seeds (McPhee, 1925 as cited in Bessey, 1933).

Using mainly feminized seed. Borthwick and Scully (1954) put hemp plants under 14, 11, and 8 hours of light, and found that between 24% and 36% of female plants produced male flowers at 8 hours and between 27% to 44% produced males at 11 hours and no males were produced at 14 hours. Through these experiments it was shown that long photoperiods inhibit sex reversal in hemp plants.

Rejuvenation

Clones can be taken from flowering plants through a process called rejuvenation. Rejuvenation is the process of a flowering plant returning to the vegetative stage by sending up a second vegetative shoot after flowering (Schaffner, 1921). If a favorable plant is found after the onset of flowering a group of clones can be taken from the plant and rejuvenated preventing a loss of genetic material. It can also be used to select for traits that occur after flowering.

Clones that have been rejuvenated can be grown and used in the breeding program just the same as those taken from plants while in the vegetative state. This technique is useful for breeding programs that do not have the technology for micropropagation or tissue culture. It does not cost more than vegetative clones and can be done by the simplest of programs.

Schaffner experimented with plant rejuvenation as well. He took mature flowering plants and placed them under 24-hour light. He was able to rejuvenate plants up to three times extending their life span from 15 weeks to over a year. The plants responded to only a small amount of electric lighting which kept them from dying and instead promoted new growth. The plants regrew shoots from lateral and terminal buds with alternate phyllotaxy. They grew to about three feet high and were able to go into a second period of flowering (Schaffner, 1926).

When rejuvenated the leaves of the new shoots were distorted in various ways (Figure 4). Some of the leaves had one smooth blade rather than jagged leaflets. The leaves continued to grow in various ways until rejuvenation was complete and they returned to a several jagged leaflets. The leaves after rejuvenation all started out a simple leaf with only one lanceolate lobe. As growth continued the leaves formed three to five lobes. The distortion of the leaves was more pronounced in the plants that were further into flower (Schaffner, 1926).

Figure 4 **Cannabis plants** A) A typical cloned plant B) A rejuvenated cloned plant

Conclusion

Hemp is a versatile plant with a major possible economic impact. It has many uses for each type of crop. The fiber industry has great potential as it ventures into the biofuel and construction areas. Seed production provides for human food and oils. Cannabidiol has a bright future in medical research and prospective health benefits.

Research of the Cannabis genome is far from complete. There are many unknowns and hurdles along the way. Hemp breeding has many objectives that seem feasible. It is behind the curve on the level of domestication that other crops have. More wide-spread growth and breeding programs should increase the availability of stable varieties. Legal and technical impediments due to hemps recent legalization need to be overcome to make stable strains into cultivars.

Photoperiod manipulation can be used in breeding programs by using sex reversal to create marketable feminized seed. The use of photoperiods can speed up breeding allowing for multiple generations a year. Rejuvenation can keep mother plants alive after flowering and available for cloning.

The future for hemp is bright. As more breeders and farmers join the industry it will become a mainstream crop such as corn or soybeans. Hemp could be the new biofuel producer. Houses could be made of hempcrete. CBD could very well be the new medical breakthrough. As interest in hemp grows the benefits of hemp will be brought to light.

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References

Alliance, Canadian hemp trade. 2020. Canadian Hemp Trade Alliance. http://www.hemptrade.ca/eguide.

Bakel, V., H. Van Bakel, J.M. Stout, A.G. Cote, C.M. Tallon, et al. 2011. The draft genome and transcriptome of Cannabis sativa The draft genome and transcriptome of Cannabis sativa. Genome Biol. doi: 10.1186/gb-2011-12-10-r102.

Bessey, E.A. 1933. Sex Problems in Hemp. Q. Rev. Biol. doi: 10.1086/394434.

Borthwick, H.A., and N.J. Scully. 1954. Photoperiodic Responses of Hemp. Bot. Gaz. doi: 10.1086/335843

Britannica, T.E. of E. 2019. Hemp Plant. https://www.britannica.com/plant/hemp.

Chandra, S., H. Lata, I.A. Khan, and M.A. ElSohly. 2017. Cannabis sativa L.: Botany and horticulture. Cannabis sativa L. - Botany and Biotechnology

Chandra, S., H. Lata, M.A. ElSohly, L.A. Walker, and D. Potter. 2017. Cannabis cultivation: Methodological issues for obtaining medical-grade product. Epilepsy Behav. doi: 10.1016/j.yebeh.2016.11.029.

Clarke, R.C. 1981. Marijuana Botany: An Advanced Study: The Propagation and Breeding of Distinctive Cannabis. Ronin Publishing.

Clarke, R.C., and M.D. Merlin. 2016. Cannabis Domestication, Breeding History, Present-day Genetic Diversity, and Future Prospects. CRC. Crit. Rev. Plant Sci. doi: 10.1080/07352689.2016.1267498.

Fike, J. 2016. Industrial Hemp: Renewed Opportunities for an Ancient Crop. CRC. Crit. Rev. Plant Sci. doi: 10.1080/07352689.2016.1257842.

Grassi, G., and J.M. McPartland. 2017. Chemical and morphological phenotypes in breeding of Cannabis sativa L. Cannabis sativa L. - Botany and Biotechnology

Green, G. 2005. The Cannabis Breeders Bible.

Green, G. 2017. The Cannabis Grow Bible. 3rd ed. Green Candy Press.

Hemp timeline. 2019. Manitoba Harvest hemp foods.

https://manitobaharvest.com/hemp-academy/lessons/hemp-history-timeline/.

Johnson, R. 2011. Hemp as an agricultural commodity. Cannabis Sativa for Health and Hemp

Laursen, L. 2015. The cultivation of weed: researchers are getting closer to answering the centuries-old question of how to label cannabis varieties--a necessary step to bring the plant into mainstream agriculture. Nature 525(7570).

Lubell, J.D., and M.H. Brand. 2018. Foliar sprays of silver thiosulfate produce male flowers on female hemp plants. Horttechnology. doi: 10.21273/HORTTECH04188-18.

McPartland, J.M., and G.W. Guy. 2017. Models of Cannabis Taxonomy, Cultural Bias, and Conflicts between Scientific and Vernacular Names. Bot. Rev. doi: 10.1007/s12229-017-9187-0.

McPartland, J.M. 2017. Cannabis sativa and cannabis indica versus “sativa” and “indica.” Cannabis sativa L. - Botany and Biotechnology

McPartland, J.M. 2018. Cannabis Systematics at the Levels of Family, Genus, and Species. Cannabis Cannabinoid Res. doi: 10.1089/can.2018.0039.

Mohan Ram, H.Y., and R. Sett. 1982. Induction of fertile male flowers in genetically female Cannabis sativa plants by silver nitrate and silver thiosulphate anionic complex. Theor. Appl. Genet. doi: 10.1007/BF00275107.

Parsons, J.L., S.L. Martin, T. James, G. Golenia, E.A. Boudko, et al. 2019. Polyploidization for the genetic improvement of cannabis sativa. Front. Plant Sci. doi: 10.3389/fpls.2019.00476.

Pollio, A. 2016. The Name of Cannabis: A Short Guide for Nonbotanists. Cannabis Cannabinoid Res. doi: 10.1089/can.2016.0027.

Raman, V., H. Lata, S. Chandra, I.A. Khan, and M.A. ElSohly. 2017. Morpho-anatomy of marijuana (Cannabis sativa L.). Cannabis sativa L. - Botany and Biotechnology

Salentijn, E.M.J., J. Petit, and L.M. Trindade. 2019. The complex interactions between flowering behavior and fiber quality in hemp. Front. Plant Sci. doi: 10.3389/fpls.2019.00614.

Salentijn, E.M.J., Q. Zhang, S. Amaducci, M. Yang, and L.M. Trindade. 2015. New developments in fiber hemp (Cannabis sativa L.) breeding. Ind. Crops Prod. doi: 10.1016/j.indcrop.2014.08.011.

Schaffner, J.H. 1918. The Expression of Sexual Dimorphism in Heterosporous Sporophytes. Ohio J. Sci. 18(4): 101–125.

Schaffner, J. 1921. Influence of Environment on Sexual Expression in Hemp. Bot. Gaz. 71(No. 3 March 1921): 197–219.

Schaffner, J.H. 1923. The Influence of Relative Length of Daylight on the Reversal of Sex in Hemp. Ecology. doi: 10.2307/1929179.

Schaffner, J.H. 1926. The Change of Opposite to Alternate Phyllotaxy and Repeated Rejuvenations in Hemp by Means of Changed Photoperiodicity. Ecology. doi: 10.2307/1929314.

Schaffner, J.H. 1931. The Fluctuation Curve of Sex Reversal in Staminate Hemp Plants Induced by Photoperiodicity. Am. J. Bot. doi: 10.2307/2435878.

Schluttenhofer, C., and L. Yuan. 2017. Challenges towards Revitalizing Hemp: A Multifaceted Crop. Trends Plant Sci. doi: 10.1016/j.tplants.2017.08.004.

Small, E. 2015. Evolution and Classification of Cannabis sativa (Marijuana, Hemp) in Relation to Human Utilization. Bot. Rev. doi: 10.1007/s12229-015-9157-3.

Small, E. 2017. Classification of Cannabis sativa L.: In relation to agricultural, biotechnological, medical and recreational utilization. Cannabis sativa L. - Botany and Biotechnology

Small, E., and A. Cronquist. 1976. A practical and natural taxonomy for cannabis. Taxon. doi: 10.2307/1220524.

Sterns, J. 2019. Is the Emerging U.S. Hemp Industry Yet Another Boom–Bust Market for U.S. Farmers? Choices.

Truta, E., Z. Olteanu, S. Surdu, M.-M. Zamfirache, and L. Oprica. 2007. Some aspects of sex determinism in hemp. Analele Stiint. ale Univ. Alexandru Ioan Cuza" din Iasi Sec. II a. Genet. si Biol. Mol.

Yampolsky, C. 1920. The Occurrence and Inheritance of Sex Intergradation in Plants. Am. J. Bot. doi: 10.2307/2435083.