



ADVANCING SCIENTIFIC DISCOVERY

TO BRING THE CANNABIS INDUSTRY FORWARD



Somewhere, something incredible is waiting to be known

Carl Sagan

About the Photo: While on one of his field checks Dr. Mike Ruckle was accompanied by his 4 year old daughter, who befriended one of the main observed insect pests to outdoor cannabis, fall armyworm (*Spodoptera frugiperda*)

PUBLISHER

PUREGENE AG

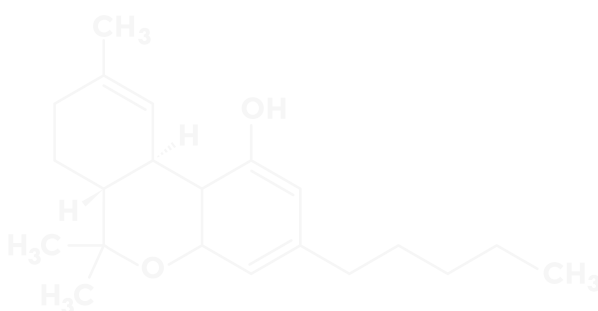
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Report by Puregene AG is
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THE ANNUAL THC EXEMPTION REPORT

With full support from the Swiss Federal Office, the 2020 Field Trial was the largest of its kind in the world and resulted in the collection of data at an unprecedented level of detail to gain critical insights into the Cannabis species. Facilitated by the most state-of-the-art methods and techniques in agriculture the results of this field trial will be a guiding example for the future of the cannabis industry at all levels. Here we highlight some of the critical discoveries we made on the field last year and how to address them for a brighter future for the industry.

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We are a next-generation breeding company with an expertise in cannabis.

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«Alone we can do
little, **together we**
can do much.»



STEVENS SENN

Chairman and Chief Executive Officer
Pure Holding AG



**dear
friends.**

What an exciting year it's been. Globally and here at home in Switzerland the opening up of markets to cannabis promises opportunities we thought impossible only several years ago. For us at PURE holding we see the unlimited potential for cannabis. We will continue to strive to ensure that the enormous benefits this wonderful plant has to offer is available to everyone – growers, producers, pharmaceutical companies, customers and patients alike.

2020 was an especially challenging year because of the COVID-19 pandemic. Adapting to these new realities at PURE demonstrates our commitment and resilience together. The past year also marked our start in working with high THC cannabis varieties. The passage of Article 8a to the Federal Act on Narcotics and Psychotropic Substances (NarcA) has effectively changed the landscape on cannabis use by allowing recreational cannabis pilot trials in Switzerland for the next 10 years for scientific study. Pure Holdings' cooperation with the BAG to trial and report on THC containing cannabis varieties has begun a process of ensuring the right approach to these varieties for Switzerland.

There is no describing how thrilling it was, after getting approval from BAG to return home from Amsterdam, entering the Zurich airport with thousands of cannabis seeds representing over 1500 different high THC containing strains. Then to realize that our

field trial, including our CBD varieties, was possibly the world's largest ever for variety evaluation and trait discovery for cannabis with 6000 unique genotypes composed of 25,000 plants. Simply unprecedented.

We see our effort to characterize these varieties as important to the Swiss cannabis economy, especially for producers and consumers, but also to support the BAG in its effort to understand the outcomes of its recreational cannabis pilot trial. In 2021, stage 2 of our THC field trial will be conducted to gain deeper insights into organic THC cultivation in Switzerland, for variety evaluation and trait-discovery.

We thank the BAG for its continued support and we look forward to working together in the future. I also want to thank the entire PURE family, whose commitment to excellence makes all this possible.

I invite you to read this report and learn more about the 2020 field trial and our mission of transformative innovation in cannabis.



«Switzerland's **unique political system** once again makes it possible for it to **play a pioneering role.**»



DR. PHILIPP RÖSLER

Member of the Board Pure Holding AG
Former Vice Chancellor of Germany



Dear friends and colleagues,

Cannabis is emerging from its history as contraband and forlorn commodity to world-wide acceptance in medicine and utility for myriad industries. Pure Holding AG is leading the way and sets the standards for an innovative and professional Swiss cannabis industry.

Innovative Switzerland has often proven that as a small country with direct democracy, in the heart of Europe, we can lead by example and open up new markets. In order to live up to its pioneering role in the European cannabis industry, Switzerland must not stand still, rather it must pave the regulatory foundations for the further growth of the industry. As it has already done in medical cannabis, Switzerland can also lead the recreational cannabis industry in Europe.

In 2020, Pure Holding received several exemption-permits from the Swiss Federal Office of Public Health for the cultivation and research of THC-containing material. This project is a synergistic collaboration between government and industry leaders with the focus on science, that will drive transformative innovation in Switzerland, and more importantly support informed decisions on cannabis regulation, which are needed for a safe and sustainable industry.

The Swiss approach to cannabis policy to first evaluate recreational cannabis use in the form of a specifically developed study is unique. If positive conclusions are drawn from the studies, which is foreseeable, leading to an open recreational Swiss cannabis market, this can have a positive domino effect throughout Europe. As with its approach to an open market for CBD, Switzerland will play a major role as a forerunner and pioneer for legalization of THC.

Our Vision - Only through scientific discovery will the true potential of cannabis be realized and it will be Puregene's scientists leading the way.



WHO WE ARE

PURE HOLDING AG

Pure Holding AG is Switzerland's premier cannabis company. It covers the entire value chain of the cannabis industry through its 6 entities - Pure Production, DEOM, Puregene, Pure Pharma, Pure Europe GmbH and Pure Europe Sàrl. A truly global company with close to 100 employees from 10 nationalities working to transform the cannabis industry. Pure Holding AG is among the largest, fully vertically integrated cannabis companies on the European market.

PURE PRODUCTION AG

Pure Production specializes in the production, processing and distribution of cannabis products. We work in cooperation with Swiss farmers in Aargau, Switzerland to produce some of the largest cannabis harvests globally. Our harvest is processed in house into our line of branded and white label products that are distributed worldwide. Large production volume and worldwide distribution has solidified Pure Production AG as a leader in the cannabis raw material trade. This product range includes flowers, various isolates, distillates, oils, cosmetics, and much more. Our entire product catalog is also available as white label products. Our partners can focus on their personal branding while we ensure a premium quality product. To better support our clients, our product development department plays an active role in the development of custom product lines for optimal product development and formulation. Our branded product line led by our tobacco substitutes; Swiss Weeds, Einhorn, Edelwiis and Alpenzwerg are distributed through over 4000 renowned retail partners making us the market leader in Switzerland. Internationally our tobacco substitutes are also available throughout Benelux countries.

PUREGENE AG

Puregene is a next-generation plant breeding company that combines Swiss quality with extensive plant science expertise and cutting-edge genomic technologies.

Puregene has built one of the largest and most detailed data sets of cannabis characteristics. This, combined with our vast genomic sequencing pipeline has allowed us to develop an unprecedented artificial intelligence-driven plant improvement program. Puregene uses predictive breeding in place of genetic modification, rather than altering the plants genetics our approach rapidly guides correct combinations of characteristics to produce varieties tailored to industry. This will fundamentally alter the way in which plants are bred. Puregene's 20-member R&D team is decoding over 300 individual cannabis characteristics that cover every aspect of its growth, development, and chemical composition. These are the building blocks for a next generation of cannabis with the potential to impact and disrupt myriad industries - pharmaceutical, cosmetic, animal feed, novel food, construction material and many more. On our journey, we aim to advance cannabis by broadly applying our genomic and artificial intelligence-driven approach to fundamentally change plant breeding. Puregene works with partners in Switzerland and globally to employ its cannabis research in support of the changing legal and health frameworks surrounding medical and recreational cannabis, to positively contribute to sustainability, to solve industrial challenges, and to educate the public, industry, and government on the potential of cannabis.



PUREGENE TEAM

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Field
Technician



ALEN OSMANBASIC

Field
Technician



LEGAL CANNABIS HAS THE POTENTIAL TO DISRUPT SOME OF THE LARGEST GLOBAL ECONOMIES AND THE PILOT PROJECTS

The potential for cannabis as an alternative to synthesized pharmaceuticals is widely accepted. Already legal, high resin cannabis is displacing the illegal cannabis market all over the world. The broad medical effectiveness of cannabis flower and active pharmaceutical preparations of cannabinoids are actively being researched, developed, and prescribed for patients suffering from a range of physical and psychological diseases. While many applications of cannabis have yet to be thoroughly researched there is a wealth of cultural knowledge suggesting efficacy in the treatment of conditions like anxiety, insomnia, PTSD, and eating disorders. Strong evidence also exists for the treatment of epilepsy, Alzheimer's, ALS, and Crohn's disease. Finally, cannabis has been shown to be highly effective in pain relief, as well as in the treatment of and recovery from heroin addiction. The seismic shift of consumer interest in THC free CBD oils, tobacco replacements, and cosmetics products has driven an incredible expansion in cannabis sales in just the last couple of years. In 2020 the global legal marijuana market was valued at over 9 Billion USD, with continued exponential growth expected as new markets open.

Less appreciated by the general public is the potential of cannabis to disrupt some of the largest global industries such as, animal feed, novel foods, construction materials, advanced and sustainable materials like foams and bioplastics with a plethora of applications. In fact, cannabis, or hemp, was once one of the most important crops grown in the United States. The fibers, which comprise up to 20% of the plant, were used in cord, sail cloth, sacks, paper, and an array of other products.

Cannabis derived products are finding applications in the cons-

truction industry as concrete alternatives, fiberboards and other multi-purpose building materials. The seeds of cannabis are an excellent source of dietary oils, Omega-3 and Omega-6 fatty acids. As an animal feed and source of high-quality protein for human consumption, cannabis seed is competitive with soybean – the most commonly used plant-based bulk protein source. Soybean, which is grown predominantly in Brazil at the expense of the rain forest, requires weekly pesticide treatments damaging the environment. By comparison, cannabis can grow well in more temperate, continental, and Mediterranean climates allowing it to be cultivated closer to production sites.

Because cannabis is an incredibly fast-growing plant that performs well in marginal conditions throughout the globe it can displace many environmentally more destructive crops and fossil-fuel based materials. Sustainable cannabis can play a major role in global climate change mitigation strategies. Today, Puregene is contributing to these efforts with research that seeks to replace advanced materials like foams traditionally produced from fossil fuels with lignin from cannabis. Cannabis based green alternatives are being shown to provide superior performance compared to their carbon-heavy counterparts.

Challenges do remain in regard to nitrogen use efficiency, but these can certainly be mitigated or overcome through modern breeding techniques. Cannabis is nowhere near its genetic potential and the technologies developed at Pure are aimed squarely at unearthing the current diversity within the species to develop the next generation of cannabis for recreation, medicine, and industry.

RECREATIONAL MARIJUANA MARKET

\$340 billion



ANIMAL FEED MARKET

\$150 billion



CARBON CREDITS MARKET

\$200 billion



PLASTICS MARKET

\$500 billion



FORESTRY AND MATERIALS MARKET

\$600 billion

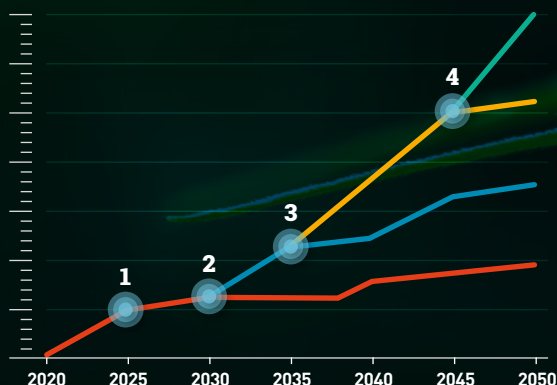


PHARMACEUTICAL MARKET

\$1200 billion



VALUE OF CANNABIS (in billions USD)



Market estimates were made by Dr. Gavin George & Dr. Michael Ruckle, based on combining traditional market research with genetic gain seen in the Puregene research program.

1. Cannabis prohibition ends in key global markets
2. Research advances in novel cannabinoid chemistry gains broad acceptance in the Pharma market
3. Hemp seed and fiber yields overtake soy and corn for protein and biofuel productivity
4. Carbon credits become a major green economy

— CARBON CREDIT ECONOMY
— FIBER, FUEL, AND FEED
— PHARMACEUTICAL
— RECREATIONAL



WHAT ARE THE SWISS EXPERIMENTAL ARTICLES & THE PILOT PROJECTS



Pure Holding AG supports the mission of the Federal Office of Public Health to pilot the recreational use of cannabis in Switzerland. We see ourselves as a natural partner to the Swiss cannabis community.

Cannabis was already cultivated in the lowlands of the Swiss Alps as early as 6,500 years ago. In the Middle Ages cannabis cultivation was widespread throughout Europe with Switzerland at the epicenter of this important industry. Cannabis was firmly anchored in Swiss agriculture and practically every Swiss farm had its own cannabis field until the international prohibition of the 20th century.

Since the introduction of the cannabis prohibition in 1951, Switzerland has had a complex relationship with cannabis. It is complex because prohibition has been proven an ineffective means of reducing the rate of use and in practice is not enforceable. At the turn of the millennium cannabis shops were to be found throughout the country. Eventually, regulators stepped in, and these were shut down. So, in Switzerland the pendulum of regulation swung in one direction or the other between regulation and prohibition. For decades, the Federal Council, commissions and NGOs have presented several variants for revising the general prohibition, but only in recent years have constructive changes towards an improvement been achieved.

The adjustment of the THC limit from 0.3% to 1% THC in the Narcotics Act in 2011 laid the foundation for a legal cannabis market long before the registration of the first CBD product. Starting in 2016 with the cultivation of resin-type cannabis strains that have a THC content of less than 1%, but still show high CBD concentrations, a cannabis boom has taken place in Switzerland which continues to this day. During the last few years, a rapidly growing and innovative legal Swiss cannabis industry has emerged, which has contributed to the acceptance of cannabis in Switzerland and Europe.

The Swiss Cannabis Medicinal Products Act will come into force in mid-2022 to regulate the medical dispensing of cannabis.

Furthermore, the cannabis pilot project is testing the dispensing of recreational cannabis in a trial setting. With this approach Switzerland is taking the right and necessary steps for a smart cannabis policy for the future.

With amendment of Art. 8 to the Federal Narcotics Law, the controlled distribution of cannabis for recreational use within the framework of scientific studies has been allowed.

The goal of the pilot projects is to create a legal basis for the implementation of scientific pilot projects that are limited in terms of location, time and scope, in order to gain knowledge about the effects of a regulated recreational cannabis market in Switzerland and its effects on the consumption behavior of participants. The conclusions of the pilot projects will then form the basis for regulating recreational cannabis in Switzerland.

- In brief, the goal is to gain knowledge and scientific insights about the effects of a regulated recreational cannabis market in Switzerland through a special study setting.
- The pilot projects should further provide insights into the effects on the physical and mental health of consumers, their performance, and socio-economic issues.
- They should also provide insights based on their effect on the black market, prevention, public order and safety.

At Pure we believe these studies will finally and scientifically show the benefits of a regulated cannabis market oriented towards the reality of cannabis consumption in the Swiss population. Rather than ignore it the focus should be on prevention, product safety and consumer protection.



WHAT ARE THE SWISS EXPERIMENTAL ARTICLES & THE PILOT PROJECTS

ROADMAP



**2019
Q1**

Dispatch on experimental articles was submitted to parliament by the Federal Council.

**2020
Q2**

National Council approved and passed on to the Council of States. Condition of organic swiss hemp added.

**2020
Q3**

Council of States agreed, but has a different opinion on the organic production and changed the condition to „if possible“.

**2020
Q3**

National Council and the Council of States were able to resolve their differences, giving the experimental article the green light.

FOPH started to draft the legislation.

**31st
March
2021**

Publication of the legislation by FOPH.

**15th
May
2021**

The amendment to the Narcotics Act will come into force. Application process for pilot projects opens.

**14th
May
2031**

**END OF
PILOT PROJECTS**

**THE ACT OF OCTOBER 3, 1951 ON
NARCOTICS² IS AMENDED AS
FOLLOWS:**

Art. 8a Pilot trials

1. After hearing the cantons and communes concerned, the Federal Office of Public Health may authorise scientific pilot trials involving narcotics with cannabinoid with cannabinoid-like effects, which:
 - A. are limited in space, time and purpose;
 - B. provide knowledge regarding the effect of new regulations on the non-medical use of such drugs and provide knowledge about the effect of new regulations on the non-medical use of these drugs and about the changes in the health status of the participants;
 - C. are conducted in a manner that ensures the protection of health and youth, the protection of public order and public safety; and
 - D. if possible, involve cannabinoid products of Swiss origin that meet the standards of Swiss organic farming.
2. The Federal Council shall lay down the conditions for the implementation of pilot trials.

2020

Q1 | Pure submits request for special permit to conduct variety and trait discovery trial within the scope of the pilot projects.

Q2 | Pure receives special permit and makes first legal seed import of 1500 THC strains to Switzerland.

Q3 & Q4 | Pure conducts the world's largest variety and trait discovery trial (25'000 Plants on the field, 5000 THC).

2021

Q3 & Q4 | Pure is going to conduct stage two of the variety and trait discovery field trial, tailored to the pilot projects.



Snezana Bajic, field technician, and Wienke Peters, breeding intern, plant seedlings for the 2021 trait discovery field trials. Without their effort our research would not be possible.



ONE MISSION TO
ADVANCE THE
POTENTIAL OF
CANNABIS FOR THE
BETTERMENT OF ALL
AGRICULTURE, FOR
THE HEALTH AND
WELLBEING OF
PEOPLE, AND FOR
THE SUSTAINABLE
FUTURE OF THIS
PLANET

THE FOUR exemption permits that enabled the THC field trials

The Federal Office of Public Health has issued four exemption permits for the import, cultivation, analysis and research on high THC producing cannabis varieties.

- 01** Exemption permit for a field trial by Pure Production AG to test varieties within the scope of the experimental article, which are optimally suited for the local environmental conditions and can be grown under organic conditions.
- 02** Exemption permit for Puregene AG to carry out research and development with over 50 different varieties with high THC content. Puregene AG has the largest THC R&D permit in Europe, which allows the group to work with an extensive gene pool.
- 03** Exemption permit to perform analysis with THC.
- 04** Import license from Swissmedic to obtain THC seeds from all over the world.



THE NEED FOR A FIELD EXPERIMENT

In the following pages of our 2020 Field Report we would like to take you through how our field trial was conducted, what we observed in the field, and highlight how Puregene is using those observations to address some of the critical issues facing cannabis cultivation in Switzerland and globally. We are pursuing this because it is essential to avoid the pitfalls of the cannabis industry now plaguing the US and Canadian markets. Much of what is wrong with the cannabis industry stems from the lack of regulation around variety standards and variety naming.

Lack of variety standards can jeopardize performance by the adoption of underperforming varieties. Some report that the Canadian cannabis industry has suffered massive losses due to the cultivation of unsellable low-quality product and because of unsustainable crop yield. In legalized markets of North America investment in cannabis has neglected better genetics in favor of creating perfect growing environments. This is a recipe for disaster. Regardless of the growing conditions, bad genetics will produce a low-quality product. In fact, 80% of all legal production in Canada is of insufficient quality to be sold, and the black market is still strong. This is precisely what we aim to avoid for the future Swiss market. Moreover, the industry is missing out on the tremendous value of the over 100 different cannabinoids cannabis produces. Instead, outdated breeding programs are still focused mostly on THC and CBD. Our THC field trial evaluated the performance of over 100 THC varieties and has carried out key experiments to determine important agronomic parameters for the cultivation of cannabis in Switzerland.

The Swiss pilot projects' requirements for outdoor organic cultivation poses a unique challenge for the production and sale of THC varieties. Most commercial THC varieties have been adapted to indoor growing conditions with automated light cycles, drip fertigation, and control over temperature and humidity. Indoor

cultivation produces superior flowers to outdoor cultivation, it prevents yield loss from weather damage and reduces pathogen exposure. Unfortunately, these luxuries have led to poor breeding practices that focus on only one or two favorable traits. They also come with a hefty price tag for both the producer and the environment, with 2% of all electricity consumption in California and Colorado attributed to indoor cannabis production. Rather than developing better performing outdoor varieties, the industry is burdened with abandoned largescale outdoor cultivation operations, overproduction of unsellable harvests, and declining revenues for some of Canada's largest producers. These are some of the challenges faced when trying to quickly and easily secure sustainable crop yields and high-quality biomass without investing in better genetics. It is clear that the Swiss pilot projects' prerequisite for outdoor organic growing will require careful selection of varieties with superior genetics that reach beyond THC and CBD content.

Varieties should be productive but also appeal to consumers who have come to expect exceptionally high-quality flowers - perfectly dense, covered in glistening trichomes, have touches of bright color, are extremely pungent with complex flavor profiles and deliver a positive experience. Global investment in cannabis has neglected better genetics in favor of creating perfect growing environments. This is a recipe for disaster. Regardless of the growing conditions bad genetics will produce a low-quality product. This is a critical issue that should be considered. In countries like the United States and Canada black market cannabis grown illegally indoors still competes with the regulated cannabis market. This could also happen in Switzerland where non-organic illegally grown cannabis has appeal. Puregene aims to put these concerns aside by selecting better varieties, innovating in organic growing and by initiating a breeding program to adapt THC varieties to outdoor organic conditions.



**FOR RESULTS OF OUR
VARIETY EVALUATIONS
OR QUESTIONS ABOUT
THE THC-EXEMPTION
FIELD EXPERIMENT
PLEASE CONTACT US AT**

info@puregene.com



HISTORY OF CANNABIS BREEDING

The prohibition of cannabis prevented it from maturing to its full potential. We only have to look to a crop like corn to see what could be possible.

Our Director of Plant Science, Michael Ruckle, often compares cannabis to a high performing crop species like corn. The comparison is, there is no comparison. While corn has undergone intensive and directed breeding to create high performance varieties, cannabis has largely suffered from neglect. Cannabis was domesticated already more than 12,000 years ago in East Asia but only recently has modern drug type- and hemp type cannabis emerged. Large scale prohibition at the turn of the 20th century

played a large role in stalling development in cannabis breeding and in shaping the present state of available varieties.

Because of prohibition much of the breeding of THC varieties was driven by pseudo-legal breeders in the Netherlands, Spain, and Switzerland, drug cartels in Mexico and Columbia, and clandestine hobby growers in Northern California selecting for flavors and better „highs“ rather than important agronomic traits. The development of better hemp varieties suffered as well. Though hemp was once grown extensively for its use in textiles, food, and oilseed it was mostly abandoned due to prohibition and displaced by cotton and petrochemical based products. It's not surprising then, that in the cannabis varieties trialed we often find unstable genetics, lack of resistance to pests, and plants not suited to the specific needs of growers and customers.



- Ancestral cannabis
- Hemp cannabis (**SATIVA**)
- Feral high-resin cannabis (**INDICA**)
- Breeding hubs for high-resin cannabis (**HYBRID**)
- Breeding hubs for hemp cannabis

Evolution of domesticated cannabis based on genomic sequencing as reported by Ren *et al.* 2021 Science Advances and Puregene's unpublished results.

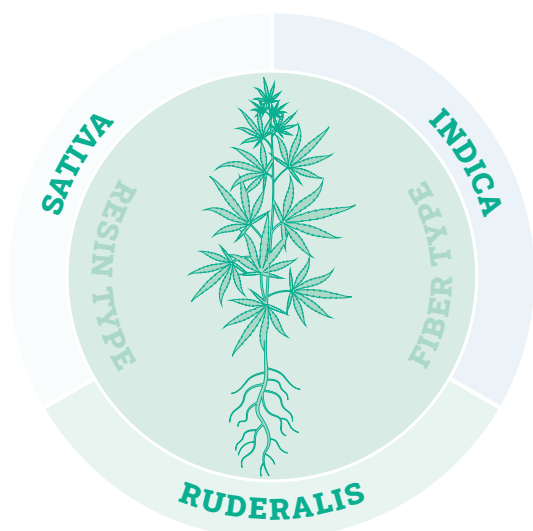


HISTORY OF CANNABIS BREEDING

INDICA OR SATIVA – Myth or Fact

Is that variety more “Indica” or is it more “Sativa”? On one hand, there is a clear ethnobotanic element that has scientific merit, such as South American shamans who prefer the psychedelic effects of “sativa” landraces for their spiritual rituals, versus Indian gurus who tend toward the relaxing effects of “indica” landraces for their spiritual rituals. This historical spiritual connection to cannabis is an important consideration when cannabis aficionados decide which variety will give them the „high“ they are looking for.

Sativa varieties are said to be tall plants with slim leaves giving the user a euphoric high. Indica varieties are shorter with wider leaves and provide a relaxed body high. Often Cannabis sativa and Cannabis indica are designated as separate species or subspecies. There is a lot of confusion and misinformation about what makes a Sativa and what makes an Indica. In reality, there is only one species – Cannabis sativa L. – that has been extensively selected for different qualities, e.g. THC amount, terpenoid profile, and fiber quality. It may be the selection for different terpene and cannabinoid concentrations that helped grow the myth of “Sativa vs Indica”. The terpenoid, myrcene, is one of the most abundant compounds found in some cannabis varieties. Myrcene, associated with so-called Indica varieties is said to cause the sedative and muscle relaxant effects, while pinene and limonene have been tied to the euphoric “high” associated with so-called Sativa strains. While the chemistry and effect of cannabinoid and terpene concentrations is highly worth investigating more carefully there is no genetic basis for naming a variety as more “Indica” or more “Sativa”.



“

Every time I hear the words “indica” or “sativa” its almost always met with a very skeptical look. The current use of the two terms in marketing of cannabis varieties is meritless, misleading, and most often meaningless. I understand the desire to connect to cannabis’ historical use, which I strongly believe exists. But until science unlocks the chemistry and genetic basis behind this mystical theory, the industry needs to focus on producing chemically and genetically stable products that consumers can reliably base their individual experiences on.

DR. MIKE RUCKLE, PHD
Director of Plant Science
at Puregene AG



WILL OUTDOOR ORGANIC PRODUCTION BE ABLE TO COMPETE WITH THE BLACK MARKET

Switzerland is the largest consumer of organic products per capita globally. In the last 20 years the number of organic farms in Switzerland has risen more than 50% to meet consumer demand driven by environmental and personal health concerns. So, it's not surprising that the recreational cannabis pilot program allows only for the production of organic cannabis. Puregene and Pure Production support the decision to only allow organic cultivation of cannabis for the Swiss pilot project. Associating Swiss cannabis quality with organic cultivation can only be an advantage to how Swiss cannabis products are viewed all over the world.

Organic cultivation methods can be challenging, but with the right know how organic agriculture can be economically and environmentally beneficial. On the economic side, income from Swiss farms practicing organic agriculture is typically 25% higher than those of conventional farmers. Environmentally, organic agriculture practices can conserve soil, support local biodiversity, and decrease agricultural runoff into groundwater. A recent survey from The Research Institutes of Organic Agriculture, FiBL, found that more than 70% of Swiss participants support the UN Sustainable Development Goals, in particular, maintaining and strengthening local and short supply chains, reducing the use of chemical-synthetic pesticides, and conserving biodiversity.

Switzerland's harsh climate and difficult terrain—small sloping fields with natural obstacles—pose unique challenges even to conventional agriculture. Puregene and Pure Production are working with our partner farmers to develop organic methods designed for outdoor cannabis cultivation in Switzerland. To date, however, there are no detailed guidelines for cultivation of organic cannabis in Switzerland, posing a challenge to ensuring Swiss farmers are compliant with the requirements of the pilot project. Puregene and Pure Production are prepared to work with the Swiss Federal Office of Public Health and Bio-Suisse to establish guidelines for the organic cultivation of cannabis in Switzerland and on standards for the import of organic cannabis.

One of the biggest challenges with organic production of cannabis is producing the same high quality flowers that consumers are accustomed to acquiring from the black market. High quality flowers are bright green, very dense, covered in trichomes, disease free, and very pungent. To obtain this quality the black market generally grows indoor with intense artificial lights and uses unregulated levels of pesticides and mineral fertilizers. Getting the right variety will be key, but realistic expectations are needed, as the current varieties have never been adapted to this type of production system.



”

Many great varieties that were bred for indoor cultivation where they are given massive amounts of mineral fertilizer and maintained with pesticides, just don't work in organic conditions. Cannabis grows well in Switzerland but we need the right varieties for outdoor organic farming. Almost all of the varieties tested underperformed and produced low quality flowers. That said, I observed several traits in the field and I believe the goal of high-quality organic production is genetically feasible, but will be a challenge.

MAXIMILIAN VOGT (PHD)

Head Breeder
at Puregene AG

Fun Fact:

Are cannabinoids a natural pesticide?

The ecological functions of cannabinoids in cannabis may include protection from the sun, from drought, and roles in plant defense. Their exact evolutionary role is yet to be understood, but it may not be to ward off hungry insects, as they are the only members of the animal kingdom without an endocannabinoid receptor system.



THE FUTURE CANNABIS INDUSTRY GETTING THE RIGHT VARIETY FOR SWITZERLAND

Cannabis as an agricultural crop is one of the most chemically and phenotypically diverse crop species utilized by mankind.

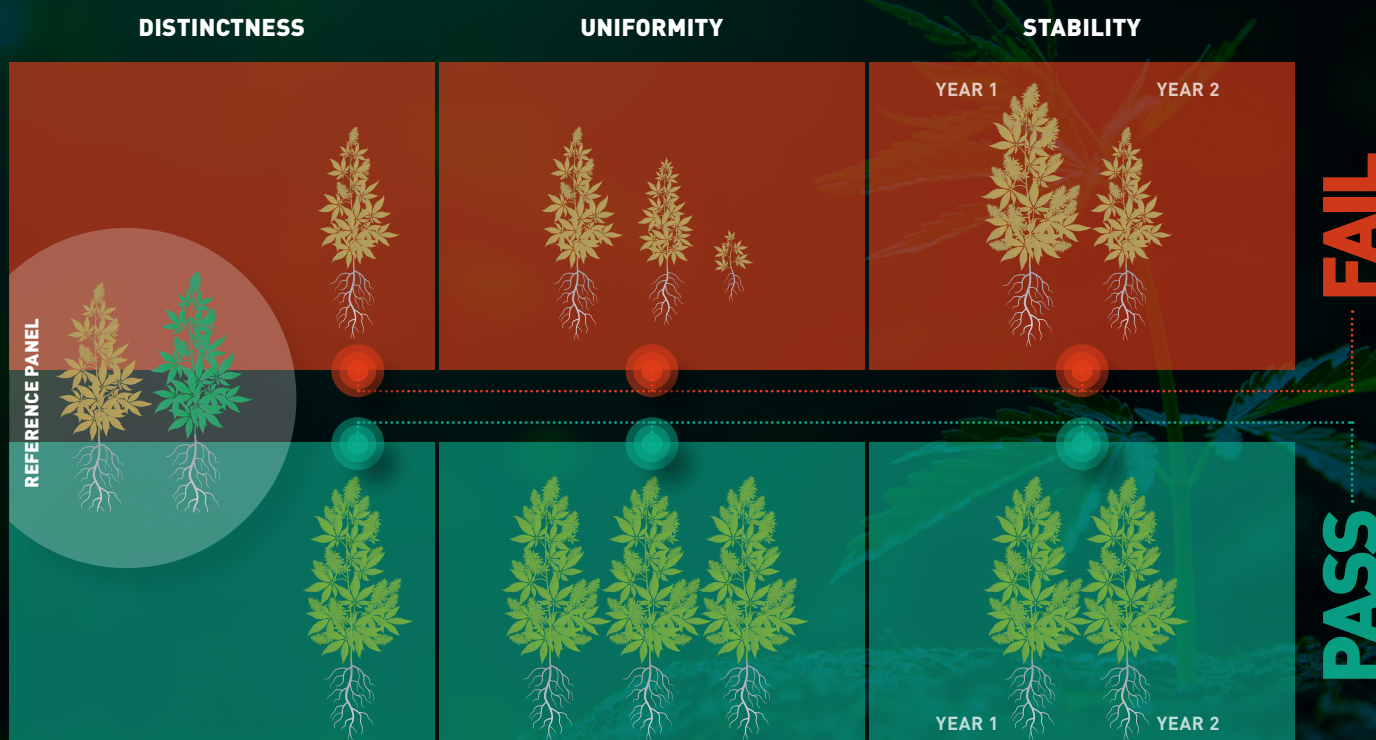
Unfortunately, the standards and regulatory practices for defining the biological and chemical variation in cannabis as an agricultural crop have not been set. Variety testing matrices that have been extensively applied to almost all crop species in the food system, such as value for cultivation and use (VCU), and distinctiveness, uniformity, and stability (DUS), have not been defined for cannabis. Variety testing protocols and variety registration are designed to protect the producer and the end consumer from fraudulent varieties. When we buy a Pink Lady apple or a Gala apple, we know what to expect because variety control for apples is highly regulated.

An important question in the young industry varieties that must be address before maturation of the market is: How do we make varieties so that the value chain can be standardized from cultivation to medicinal or recreational use? Although fiber and seed hemp have clear paths for variety testing, flower cannabis varieties are commonly distinguished using popular names with major distinctions being made with arbitrary terms of pedigree

like "indica", "sativa", "haze" or "kush". Although more than 4000 different cultivars have already been described, it is unclear whether such classification reflects any relevant differences in stable chemical composition. Currently chemical composition protocols have only been applied to define cannabis varieties for forensic applications to distinguish drug varieties from non-drug hemp varieties. Moreover, the bioactivity of over 100 terpenoids present in cannabis only makes the proper definition of varieties more complex. As the industry matures to bring the benefits of cannabis to the market it is clear that a better classification system for varieties is needed.

There are several studies that look at the chemical diversity in the cannabis species. These projects are generally small and carried out in secure indoor facilities. Due to historically restrictive legal requirements placed on cannabis research, few studies have been carried out to a scale that is needed to understand the agronomy and horticulture for professional production of cannabis. Even less is known about how cannabis varieties perform in outdoor settings. Outdoor production of multiple varieties is the missing experiment required to bridge the gap between small scale indoor studies on cannabis chemistry and production required by the industry.

HOW VARIETY TESTING WORKS | DUS BY MORPHOLOGICAL TRAITS





WHY IS DEFINING A VARIETY IMPORTANT?

The standards and regulatory practices for defining the chemical variation in cannabis as an agricultural crop have not been established.

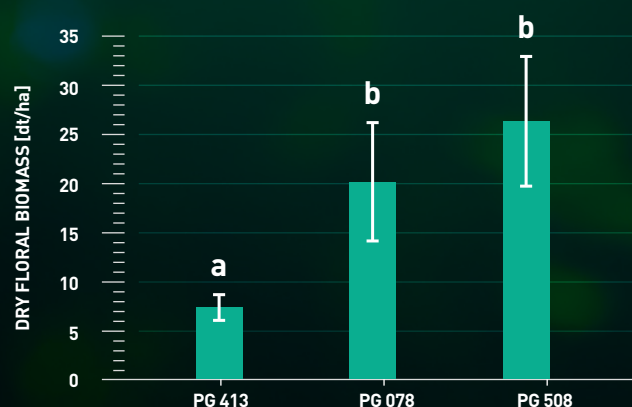
In other crops, variety testing matrices have been extensively applied, such as value for cultivation and use (VCU), agronomic characteristics like disease resistance, and distinctiveness, uniformity, and stability (DUS), which describes the morphology and composition of a variety. These variety testing protocols and variety registration are designed to protect producers and consumers from fraudulent varieties. DUS standards are already in place for flower cannabis in Europe at Naktuinbouw (the Netherlands Inspection Service for Horticulture), and Puregene scientist have already had more than one variety approved for DUS standards. Additionally, Puregene is working with Agroscope, The Swiss Confederation's centre of excellence for agricultural research, to define VCU and establish guidelines for cannabis regulatory standards. The aim of this project is to lay the foundational research needed to pave the way for variety registration within Europe.

It's important for the value of cannabis that producers and consumers can connect a variety's name with quality and consistency. As the industry matures, to bring the benefits of cannabis to the market a better classification system for varieties is needed. An alternative, DNA-fingerprinting offers the most reliable approach that can tell varieties apart through subtle unique genomic differences. We investigate the challenges and advantages of these approaches in the following page of this field report. Puregene recognizes the importance of combining these approaches, DNA fingerprinting, and cannabinoid and terpene composition to better define cannabis varieties for a range of applications, particularly in the absence of regulatory standards for flower cannabis from the European agriculture departments.

As mentioned, chemical composition is one way that has been used to discern drug varieties from non-drug hemp varieties and can be extended to differentiate flower cannabis varieties. Chemical composition can be affected by growing conditions, so a combination of chemical and DNA fingerprinting would offer consumers the best guarantee of product consistency and reproducible effect. Having a reproducible effect is critical for medicinal applications.



The 2020 field trial pictured above and located in Reckenholz, Switzerland was a collaborative project between Agroscope and Puregene. The project defined protocols for multiple key traits, such as dry floral biomass, and identified clear parameters that define value for flower cannabis. Defining valued traits is a first step to variety registration in the EU.



The traits that are assessed normally in standard VCU trial could be easily applied to characterize flower cannabis plants and enabled a distinction of the varieties.

DR. JÜRGEN HILTBRUNNER, PHD

Research Group Leader for
Varieties and Production Techniques



GROWING AND HARVESTING MILLIONS OF DATA POINTS

1

SOIL PREPARATION

Soil preparation



Puregene works with farmers who take organic farming seriously. Preparation for the field trial started seasons before with rotation of the field out of cultivation of a meadow of forage grasses and clover. The meadow was ploughed, then harrowed to break the soil.



The field was laid out in 31 rows covered in PLA bio-plastic foil. Each row was spaced 1.5m apart. 10-15cm under the foil drip tube lines were run for irrigation. A legume mixture was planted between rows to control weeds and provide a natural source of organic nitrogen.

2

SECURING THE FIELD

Securing the field



- Field location was tightly controlled and not visible from a main street.
- A double security fence with 1-meter gap surrounds the field.
- Cameras with thermal and night vision capability monitor continuously.
- Sensor triggered alarms alerts responsible personnel and engage security protocol.
- During harvest there was on-site security.
- All sensors and video cameras were equipped with manipulation alarms.

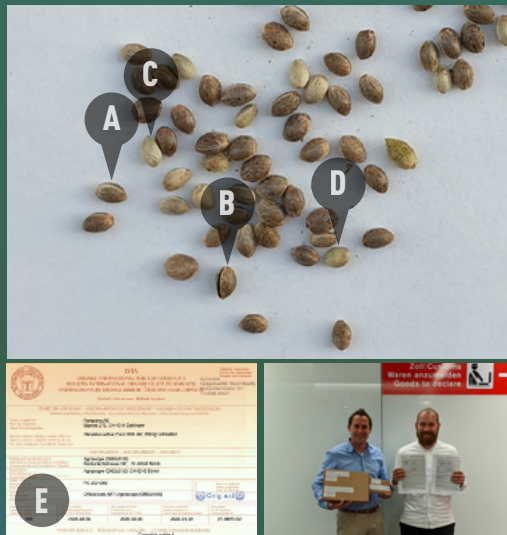


GROWING AND HARVESTING MILLIONS OF DATA POINTS

3

SEEDS

Seeds



Seed quality is important to consider. We observed three major problems within the varieties we tested:

- A. High quality seeds.
- B. Open hulls.
- C. Aborted or empty hulls.
- D. Unripe or green seeds.
- E. In traditional crops seed quality controls exist like International Seed Testing Association (ISTA) certification to protect farmers and producers. ISTA certification of Puregene CBD-seeds. Although the Swiss office in Agroscope has active protocols for hemp seed quality, the protocols for dealing with seeds that are controlled under narcotic laws will need to be addressed.

July 7th, 2020 – Stevens Senn and Lino Cereghetti after they successfully and legally imported thousands of cannabis seeds at the Swiss customs in Zurich Airport. The information in these seeds will advance cannabis research for all applications.

4

PLANTING

Planting

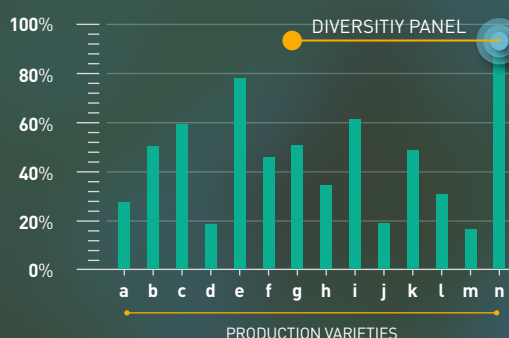


Germination

Seeds were germinated in our secure facility in Zeiningen before being transferred as seedlings to our high security field. Seeds were planted directly in a mixture of coconut fiber: perlite mixture (5:1), 2cm deep and covered in vermiculite. After 2 weeks of growth they were transferred to the field.

Germination rates of the tested production varieties varied dramatically between 20-80% due to poor seed production quality or poor breeding. Germination rates for most of the tested seeds in the diversity panel were above 80% and averaged 90% germination.

GERMINATION RATE





GROWING AND HARVESTING MILLIONS OF DATA POINTS



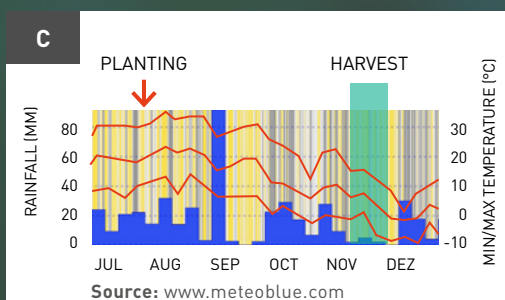
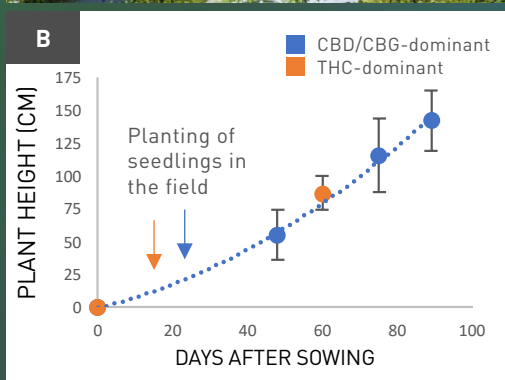
Planting

2' 685 plants with more than 1% THC were planted for agronomic testing. Another 2' 400 plants with less than 1% THC were planted as controls. The planting distance between plants within a row was 0.8 meters. Each plant was tagged with a unique germplasm identification number (GID) and QR-code. That was used throughout the experiment for data tracking

5

GROWTH

Growth



A. Field maintenance

1-9-2020. Once during the growing season liquid manure was delivered directly through the watering tubes. Throughout the trial weeding was a significant problem. Weeds were pulled by hand every 10 days.

B. Vegetative growth

In parallel to the field of the exemption permit Puregene carried out an experiment with CBD/CBG dominant varieties. Although planted 25 days later in the greenhouse and 10 days later in the field, the THC plants had the same growth rate. In general growth rate was more consistent between varieties in the THC-dominant field, as indicated by the smaller error-bars, which indicate the amount of variation in plant height.

C. Weather

The biggest challenge with farming and outdoor growth is the weather, especially for a plant that has been primarily bred in clandestine indoor breeding programs. For 2020, the summer was warm, sunny and had good rain, all perfect for cannabis. Storms at the beginning of September, caused severe lodging for cannabis planted before the middle of July, pointing out that timing of planting is critical. October was cloudy with few dry days. The lack of sun and warm days in October overlapped with flower maturation, leading to poor flower quality.



GROWING AND HARVESTING MILLIONS OF DATA POINTS

6

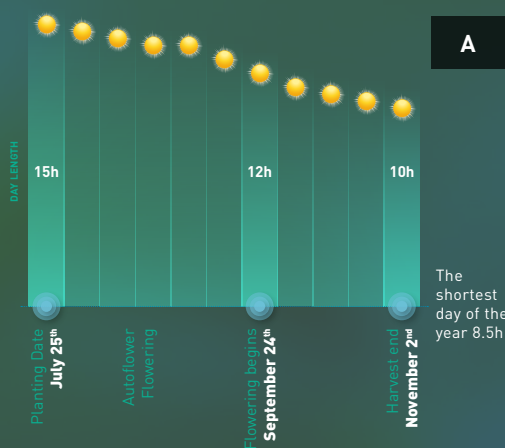
FLOWERING



D./E. Problems during vegetative growth

Broken branches and lodging were two major problems that occurred during vegetative growth, and both can lead to catastrophic losses for producers. Both traits were prevalent within specific varieties and have a genetic basis. In those varieties branches break off at the node between the branch and the main stem (d). Lodging generally occurred when secondary roots break at the node of the primary root (e). Both traits resulted in biomass on the ground that created a reservoir for fungal pathogens that infected the rest of the plants in the field. Varieties that carry these two undesirable traits are unsuitable for Switzerland.

Flowering



A. Photoperiod dependent flowering

The growing season for most varieties of cannabis is dependent on seasonal cues at the end of summer to start flowering. For most plants the main cue is day length as most cannabis varieties are short-day flowering plants. Temperature, growth stage, and stress have also been reported as flowering cues, for plants that are less sensitive to photoperiod. We planted seedlings in the field at the end of July. This allowed two months of growth before flowering began.

B. Identifying flower initiation

One of the most critical traits that was monitored was flower initiation. This trait determines photoperiod timing and is the starting point for flower maturation period. Flower initiation was documented weekly when the first stigmas (black arrow) appear out of the apical inflorescence. Some plants were male or produced anthers, which could lead to a catastrophic loss in flower quality and risks pollen contamination (see page 35).

C. Photoperiod independent flowering

Some varieties, known as autoflowers, have a trait thought to have come from the ruderalis subspecies that allows flowering in long days. The autoflower varieties in the THC experiment started flowering two to three weeks after planting.





GROWING AND HARVESTING MILLIONS OF DATA POINTS

7

HARVEST

Harvest



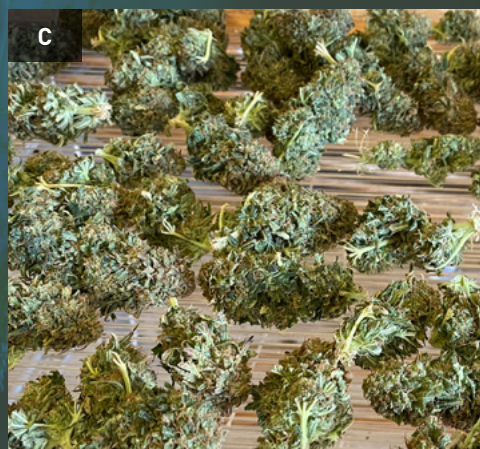
A. Selecting plants to harvest

Each plant was monitored daily during harvest. Ripe plants were harvested when flowers were evaluated to be mature by visual inspection when 50–80% of pistils had attained an amber color. Harvesting occurred on a plant-by-plant basis over a period of two weeks ending the second week of November.

B./C. Data collection, flower processing and drying

At time of harvest, general agronomic traits were assessed through image analysis. The apical flower panicle (top bud/cola) was harvested, cut to 40cm, mechanically trimmed, and frozen (b). The remaining flowers on the plant were mechanically trimmed and stripped from each branch by hand. Flower biomass was air dried on nets for 7–10 days (c).

Nearly 16 percent of plants did not initiate flowering or flower sufficiently to be considered for harvesting. Biomass that was not dried for evaluation was shredded and composted back into the field. In the end, only 62% of the germinated seeds of our trialed high-THC varieties made it to final harvest.





THE RESULTS OF 2020 THC-PILOT PRODUCTION PROJECT

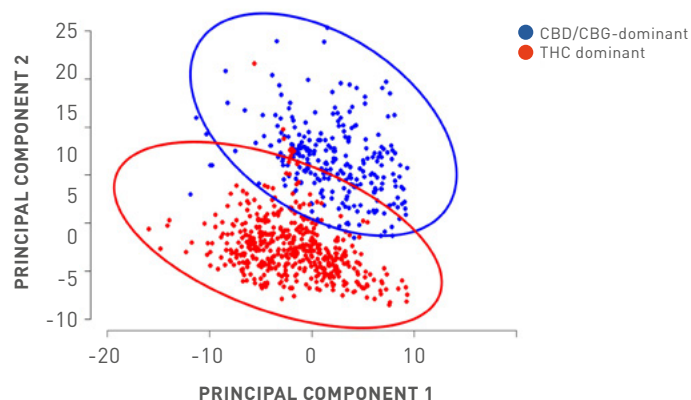


THE NEED FOR PLANTS ABOVE 1% THC IN CANNABIS RESEARCH

One of the largest open questions in cannabis research is “How much genetic potential is locked in high-THC or regulated cannabis?”. One of the goals of this field experiment was to address that exact question. One hypothesis is that since the discovery of high-resin-type CBD-dominant cannabis varieties 20 years ago, this trait has been introgressed into many different classic THC genetics. The second hypothesis is that the combination of the recently discovered CBD-dominant high-resin varieties and cannabis prohibition on breeding programs has created a wall that prevents gene flow between prohibited resin-type varieties and permitted resin-type varieties.

To address this question, we analyzed 737 unique regions on the genome that discriminate each plant at the genetic level. To put this into perspective a human paternity test uses 15 DNA regions. We tested 287 different low-THC plants against 960 diverse high-THC plants. We found that THC-dominant plants and CBD/CBG-dominant plants are genetically distinct, and there is very little overlap between the genetic sequences of the THC-dominant plants and CBD/CBG dominant plants. Therefore, prohibition has been successful at preventing gene flow into the legal market, and there is a vast unexplored gene- and trait pool locked in regulated varieties. Ultimately, the use of the diversity locked in THC varieties will increase our resources for genomic analysis, breeding, and discovery. Only through research exemptions like this one can this diversity be explored.

THC and CBD/CBG varieties are two clear genetic pools



Principal Component Analysis (PCA), is a method that reduces large complex datasets into two dimensions. With the case of genetic diversity, we use DNA markers to represent a unique variable that contributes to the variation found across and between plants. PCA analysis breaks genetic data down into smaller components. In the plot above, each dot represents an individual plant. **This plot works like a map:** the closer the dots are on this map, the more similar they are, and the more distant the more different they are. The separate grouping of THC dominant and CBD/CBG dominant plants indicates that there is a clear difference in their genetic make-up (either THC or CBD). The fact that these two clouds have very little overlap means that there has been very little cross-breeding and gene flow between CBD/CBG-dominant- and THC-dominant varieties.

WHAT IS IN A NAME?

IF YOU FIND NAMES OF CANNABIS VARIETIES CONFUSING, YOU ARE NOT ALONE. AND ITS VERY LIKELY THAT THE NAME MEANS LESS THAN YOU THINK.



Cannabis varieties bred for high THC content go by some wild names. Names developed by hobby and semi-professional breeders capture the essence of a variety but not much more. Some names describe the psychoactive effect like Haze. Others, like Diesel and Lime highlight the smell and terpene profile. Some varieties are even named for their origin like Cali Gold or Afghan Kush, while others allude to potency like Train wreck. These hazy descriptions fall short of accurately defining varieties for farmers and recreational users. Even more problematic is that genetically distinct varieties often share the same or similar names. In cannabis, naming is like the „wild west”—anything goes.

Ultimately, as there are no regulatory bodies in Europe that ensure variety standards for the consumer, producers can call almost any product what they wish. For example, if you look at a classic variety like White Widow, which is over 40 years old, there are multiple phenotypes advertised for what should be the exact same variety. It's likely that the original variety has been long lost and many of today's White Widow seeds are knock-offs made by amateur breeders. In fact, studies have shown that flowers bought in Amsterdam coffee shops named White Widow had very different chemical profiles and some flowers were more like Amnesia flowers than other White Widow flowers¹. We observed similar findings in the field experiment and found that there are a lot of differences depending on the breeder and source of the seeds.

Our Director of Research and IP, Dr. Gavin George, confronts the issue of naming varieties constantly. He acknowledges that good names are part of the marketing of a variety, but this marketing often comes with misinformation: „We need to do better. There must be some standardization, otherwise it hurts industry and the consumer in the long run. Take the apple for example, when I buy a Pink Lady or a Gala, I get the right apple every time, and an apple is not prescribed as a powerful medicine. In cannabis, I have my doubts. We have to do better. Currently it's like a box of chocolates, which is unacceptable when we think to future medical applications for cannabis”.

Although naming a variety brings a sense of pride to the breeders and researchers, akin to an artist signing a painting, Puregene's scientists are in agreement that with the current state of the industry, better solutions are needed. As a team of experts in molecular breeding, the Puregene scientists see the easiest solution to ensure the consumer gets exactly what is advertised, is to use a simple database that connects each plant's DNA fingerprint with its phenotype. This would ensure that breeders, producers, law enforcement and regulatory agencies, medical doctors, retailers, and consumers are all on the same page and know exactly what they are getting. With the advent of next-generation DNA sequencing such solutions are a cost effective and feasible solution for a big problem in the industry.



THE AWESOME POWER OF NATURAL GENETIC DIVERSITY

GENETIC DIVERSITY

The start of any successful breeding program is assembling a genetically diverse collection of cannabis varieties. Genetic diversity is the basis for crop improvement, providing the genetic resources to develop new and improved cultivars. The genetic differences that generate this diversity are important in conferring valuable traits like disease resistance, unique cannabinoids, diverse terpene profiles, flowering time, and growth pattern.

Research scientist and expert in quantitative genetics Dr. Claudio Cropano explains: „In order to breed better cannabis the more genetic diversity we have in our program the more traits we have in our toolbox to create new varieties. We suspect that THC varieties are a rich source of genetic diversity that will complement our existing collection that has untapped potential. We envision utilizing the genetic diversity we've accumulated for developing next generation cannabis varieties, with the right combination of traits to meet the needs of the industry. From what I see in the lab and the field the trait diversity is really a geneticist's playground. The next few years will be fun but challenging“.

As part of our field trial, we determined the genetic diversity of varieties using a DNA fingerprint of 5500 genomic regions; this fingerprint is 350 times more differentiating than the human paternity test. What we found was that the genetic diversity in the THC material tested was distinct from the genetic diversity of the CBD/CBG material, thereby potentially increasing our resources for genomic analysis, breeding, and discovery. “The genetic diversity was also clear to the eye. Of the plants tested in the diversity panel they were for the most part very distinguishable, and in the fall the diversity in colors and smells was truly impressive.” – Dr. Cropano noted.

Dr. Cropano and his team are working to turn the vast genetic diversity of the cannabis plant into molecular tools that Puregene's breeders can use to more rapidly select and develop new varieties. This understanding of cannabis genetic diversity is at the heart of Puregene's core values, which is discovering how nature works to benefit humanity. Part of our core mission at Puregene is to uncover the genetic basis of how cannabis works, in order to harness its potential.

The presence or absence of a particular trait can be correlated with differences in a plant's DNA, allowing us to identify the specific gene or genes responsible. Once the DNA region is identified new varieties can be assembled at the DNA level using bioinformatics and machine learning. Dr. Cropano's research is key to understanding the molecular basis of many of cannabis's most important agronomic traits. This understanding is critical to the future of cannabis breeding.



Dr. Claudio Cropano's team connects agronomic traits to the DNA. One of the clear signs of genetic diversity in the THC field was observed in the colors of the flowers. Diversity in leaf shape, leaf number, stigma color, leaf color, and calyx color combined to put on an impressive display of nature's power in the autumn.



PLANT BREEDING: THE ART OF BALANCING GENETIC DIVERSITY AND GENETIC STABILITY

Genetic stability – a breeders thumbprint on the genome

Almost every fruit, vegetable, bean, and grain tastes the way it does, not because nature provided it that way, but because it was specially selected by a breeder's hands. Cannabis is not different, although due to the clandestine nature of most cannabis breeding programs, the regulatory checks and balances that ensure quality breeding do not exist. Therefore, one aim of the field experiment was to evaluate the current state of cannabis breeding.

Plant breeding is a continual process of adding diversity in the form of desired traits and stabilizing those traits into a uniform and novel variety. The process of adding trait diversity followed by cycles of stabilization leaves a unique fingerprint on the genome of a variety. To the untrained eye it looks like noise, but Puregene's Head Breeder, Dr. Max Vogt (PhD), can see the history, pedigree, and the precision of the breeder who developed each variety he tested. Max Vogt carried out his Doctoral research on developing the DNA tools and strategies needed to maximize hybrid breeding in plants and is an expert in *heterosis.

Dr. Vogt explains: „The genetic diversity differences we found in plants of the same variety were often highly variable. This genetic instability is a product of the way these varieties have been created. Unfortunately, this poor breeding often results in growers getting inconsistent results or having to spend resources on removing unwanted, unstable traits like we see for male flowers. Growers want uniformity. A field where all the flowers are ready for harvest at the same time and have a consistent cannabinoid composition is of far more value than one that is a mess of different growth patterns and inconsistency. What we are finding is that many of the high-THC cannabis varieties are unstable at the DNA level and this instability carries over their phenotypes and

chemistry profiles. Diversity in flower color is great, but not when it's in the same variety; there I want to see consistency.”

Vogt's team analyzed heterozygosity of different varieties (as seen on p32). They looked how each part of the genome was inherited in every plant on the field. From each DNA sequence the team can determine how similar each parent is. The more similar the parents' DNA, the more similar the offspring's DNA, a concept called homozygosity. More homozygous varieties are generally more similar in appearance and chemistry. The greater the number of differences the more heterozygous a variety. You can see that some varieties showed a consistent level of heterozygosity, represented by small boxes while many varieties were highly variable in their degree of heterozygosity represented by the tall boxes. The tall boxes are a sign of genetic instability and poor breeding. This variability is troubling and indicates the genetic instability of many varieties of available cannabis.

Often plants within a variety deviated greatly in genetic similarity—these are represented by the distinct points above or below the boxes. It is likely that these outliers within varieties may be the result of pollen contamination. Pollen contamination is a common problem in cannabis breeding programs. Another potential problem is when breeders select plants that are too homozygous because they can suffer from #inbreeding depression.

As an expert in hybrid breeding, Dr. Vogt sees this as the starting point of his line breeding program, where he is developing inbred parents to make hybrid varieties, which are the breeder's holy grail of uniform heterozygosity, which is what we see in crops like maize.

Glossary:

Heterosis - The phenomenon that offspring from two diverse parents of a species or crosses between species exhibit greater biomass, speed of development, and fertility than both parents.

Inbreeding depression - is the reduced survival and fertility of offspring of related individuals.



PLANT BREEDING: THE ART OF BALANCING GENETIC DIVERSITY AND GENETIC STABILITY

Breaking down the data

Stable varieties

Genetically stable varieties have a similar degree of genetic differences, illustrated in this figure as small boxes with little variation (short vertical lines) and no outliers (single dots).

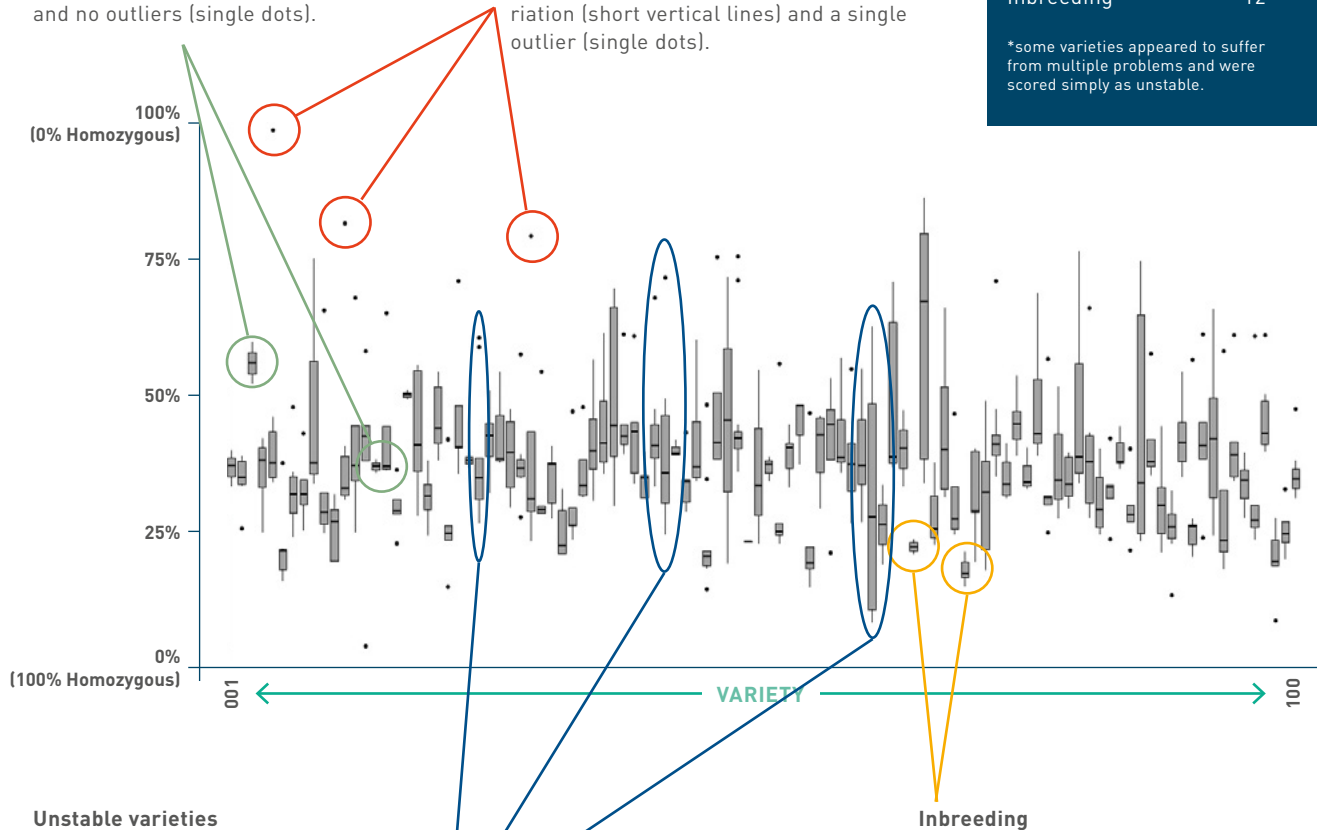
Poor pollen control

Genetically stable varieties with single outliers are a sign of pollen contamination during seed production. Illustrated in this figure as boxes with a little variation (short vertical lines) and a single outlier (single dots).

Assessed by the breeder

	Number of varieties
Stable	20
Unstable	51
Poor pollen control	9
Inbreeding	12

*some varieties appeared to suffer from multiple problems and were scored simply as unstable.



Unstable varieties

Genetically unstable varieties differ in the degree of genetic differences in each plant of a variety. Some plants in the variety are very heterozygous and some plants are very homozygous. Illustrated in this figure as tall boxes with a lot of variation (long vertical lines) and one or more outliers (single dots).

Inbreeding

Although genetically stable, varieties with less than 25% heterozygosity run the risk of inbreeding depression and can run the risk of being more susceptible to disease. Inbreeding is illustrated in this figure as small boxes with little variation (short vertical lines) and no outliers (single dots) that are all below 25% heterozygosity.



CHEMICAL STABILITY - ON THE PATH TO DELIVER CHEMICAL PRECISION.

A pharmacy or a street dealer? Getting cannabis chemistry under control.

The cannabinoid THC is the main psychoactive compound found in high-resin cannabis. But how variable is the THC concentration in flowers from different plants of the same variety? This important question for regulators, for farmers, and for consumers is rarely addressed. Regulators need assurance that THC concentrations in varieties are stable. Farmers can be in violation of the law if the cannabis they are growing is not what they thought it was. For example, varieties with THC concentrations above 20% are not allowed in the Swiss cannabis pilot project. This is not only a Swiss issue, in countries around the world legal thresholds for THC are even lower. Consumers should be able to trust the claims made by suppliers. For potential medical applications of cannabis flower, consistency needs to be as precise as pharmaceutical products are.

Do the available cannabis varieties deliver?

Dr. Daniel Carrera Head of Genomic Research: „This is a tricky question because the variables affecting outdoor organic cannabis production are many. We looked first at the overall THC concentration in dried harvested flowers in our 2020 THC field trial.”—Dr. Carrera’s group can accurately and precisely profile up to 14 cannabinoids at once with a relatively high throughput in their analytics lab—“Looking specifically at THC, the majority of varieties evaluated in our 2020 THC field trial had THC concentrations ranging from 15% to under 5%. We did find some plants having THC concentrations as high as 17% and as low as 1%. No

variety or individual plant in our field crossed the 20% threshold.”

Only about 14% of the varieties we evaluated were stable from plant to plant, having deviations in THC concentration under 1%. The rest of the varieties showed varying degrees of consistency. The worst varieties, about 19% of them, can be best described as having completely random amounts of THC content in their dry flowers. While there are differences in THC content depending on how and where you sample from the flower, with our method we saw less than 5% deviation in clonal cannabis varieties, which is incredibly good for a biological system.

Dr. Carrera’s group connected the variability of many of the varieties in this trial to their underlying genetic instability. This instability is generally not observed in the CBD cannabis industry, where clonal plants are used to produce flowers. Clonal plants are genetically identical to the mother plant that they are propagated from. In the THC field experiment we observed that cannabis plants of the same variety often had very distinct genetic signatures and it’s not surprising that the cannabinoid content in these varieties differ to the degree we observed.

Dr. Vogt is working with Dr. Carrera to develop a new standard for a consistent product and are approaching this problem in two ways. The first is identifying the most stable varieties with desired chemistry and to stabilize this chemistry at the genomic level using DNA marker-assisted breeding. The second approach is more direct; when plants are discovered to have the right chemistry, they are propagated to make genetically identical clones for production.



I was really surprised with the variability of THC content in plants of the same variety that were even grown in the same field. Growing these varieties for a specific THC outcome seems closer to roulette than medicine or science.

DR. DANIEL CARRERA PHD
Head of Genomic Research
at Puregene AG





Here Team Leader, Dr. Julian Koschmieder and Analytics Specialist, Dr. Martina Zanella, prepare one of over 10'000 cannabis samples that were analyzed by UPLC analysis to measure cannabinoid content.

Weak

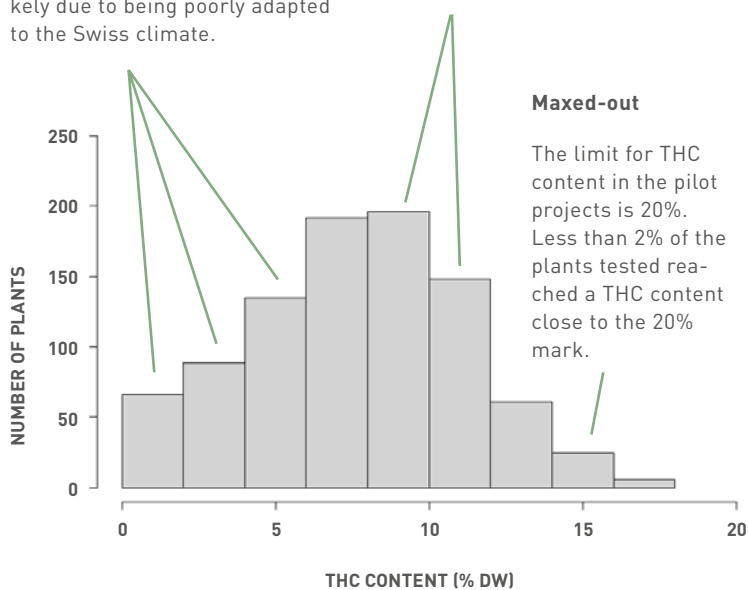
Of the varieties tested almost 1/3 of them contained 5% or less THC in their flowers. Such flowers are undesirable and likely due to being poorly adapted to the Swiss climate.

Mild

The most common phenotype observed in the tested flowers was a THC content between 8-12%.

Maxed-out

The limit for THC content in the pilot projects is 20%. Less than 2% of the plants tested reached a THC content close to the 20% mark.

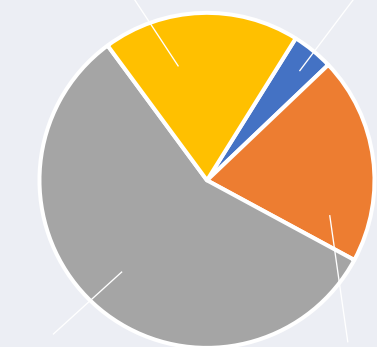


Recreation grade

20% of the varieties tested had consistency that deviated less than 25% of the mean concentration.

Pharma grade

Barely 4% of the varieties tested had consistency that deviated less than 10% of the mean concentration.



Poor

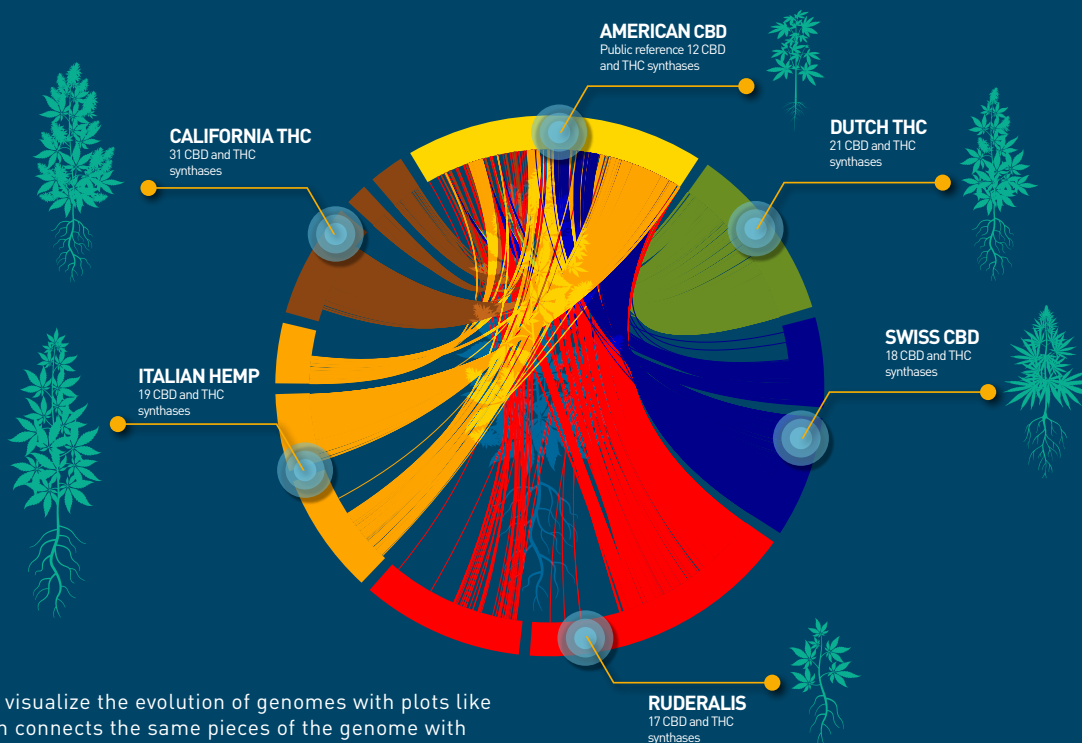
57% of the varieties tested had consistency that deviated less than 50% of the mean concentration.

Random

19% of the varieties tested had consistency that deviated more than 50% of the mean concentration.

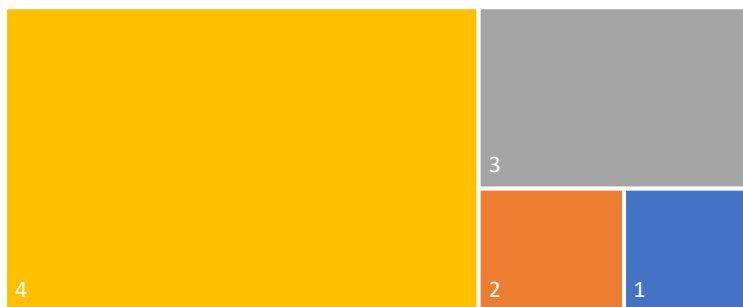


WHY SOME PLANTS MAKE THC, CBD, OR BOTH



Scientists visualize the evolution of genomes with plots like this, which connects the same pieces of the genome with ribbons. This is the region of the cannabis Pangenome where the cannabinoid synthases are located. The twists in the ribbons mean the genome has changed and evolved, these changes impact the THC and CBD contents in the flower.

THC to CBD ratios observed in the field



Above 20:1 THC:CBD
336 plants tested had a THC content that was more than 20 times higher than the CBD content in the flower.

1:1 THC:CBD
42 plants tested had an equal or 1:1 ratio THC to CBD chemotype. 19 varieties displayed this chemotype, but only 2 varieties displayed it consistently.

Above 1:1 THC:CBD

114 plants tested had a THC dominant chemotype that was above a 1:1 THC:CBD ratio but below a 20:1 THC:CBD ratio.

Below 1:1 THC:CBD

37 plants tested had a CBD dominant chemotype that was below a 1:1 THC:CBD ratio. 13 varieties displayed this chemotype but only 2 varieties displayed it consistently.



THE CANNABINOID SYNTHASE - A CENTRAL INNOVATION IN CANNABIS

A complex metabolic network lies behind the synthesis and production of cannabinoids in cannabis. Different enzymes called synthases are responsible for the production of either CBD or THC from a precursor cannabinoid known as cannabigerol (CBG). When we think about cannabis that produces very low amounts of THC and high amounts of CBD, it can be because the synthase for THC is less active, missing or because there is much more of the synthase for CBD. Subtle differences in the behavior of the synthases produces varieties with an incredible diversity of not only different THC to CBD ratios but differing amounts of all the cannabis cannabinoids. Understanding the chemistry of cannabis is very much connected to understanding the network and activity of the cannabinoid synthases.

Dr. Daniel Carrera, Head of Genomic Research at Puregene is investigating the broad and diverse family of cannabis synthases and how they relate to the cannabinoid content of distinct varieties. He explains: „In the genome of cannabis we can identify the CBD and THC synthases and synthases that are similar to these. What we are finding is that in distinct varieties the number of genes encoding CBD and THC synthases can vary extensively, as can subtle variations in the synthases themselves. These changes at the genomic level in different varieties are the result of ge-

nomomic rearrangements and duplications. Duplications can double or more the number of a gene and then create the possibility that the new duplicated gene may acquire a new function. This can have a huge impact on the diversity of different cannabinoids we find and on the cannabinoid ratios.”

To study this more closely Puregene has assembled a cannabis pangenome from the genomes of many cannabis varieties. This allows us to identify novel synthases that may be involved in the production of rare cannabinoids and provides a way to better understand the diversity and evolutionary history of synthases at the genomic level.

It's important to remember that the number of synthases and other cannabinoid-producing enzymes are not solely responsible for the concentrations of cannabinoids found. Environmental effects like drought, light, and heat can impact the final cannabinoid concentration in flowers. Traits that are not directly linked to the cannabinoids can be equally important for cannabis to achieve its full yield potential. That is why it is important that breeders select for good agronomic traits as well as cannabinoid concentrations.

Fun Fact:

Dronabinol (Marinol), is a synthetic THC drug that received FDA approval in 1985 for treatment of pain and loss of appetite. It is pure THC. It lost popularity, due to the anxiety as a common side effect. In 2010, Nabiximols (Sativex) was approved for treatment of pain by the UK-MHRA. It contains an almost 1:1 ratio of THC and CBD and has been reported to have fewer problems with anxiety compared to Dronabinol.





CHEMICAL DIVERSITY: THE CHALLENGE OF UNKNOWN COMPOUNDS IN UNDEFINED VARIETIES

Looking out over our field of high-THC cannabis is like looking over a field of bright green pharmaceutical factories. The chemical diversity of cannabinoids alone that cannabis can produce is simply extraordinary. A particular variety might be THC rich, while another might be abundant primarily in CBD. Cannabis sativa is the only plant species that produces multiple cannabinoids. Its closest relative, hops, produces cannabigerol (CBG). Over 150 different cannabinoids have been identified in cannabis, but it's not clear how many of them will be at meaningful enough amounts to be pharmaceutically relevant. Many cannabinoids only exist transiently or at low concentrations when compared to THC and CBD. But in some varieties these minor cannabinoids are more enriched and new research is showing these novel cannabinoids might be future pharmaceuticals.

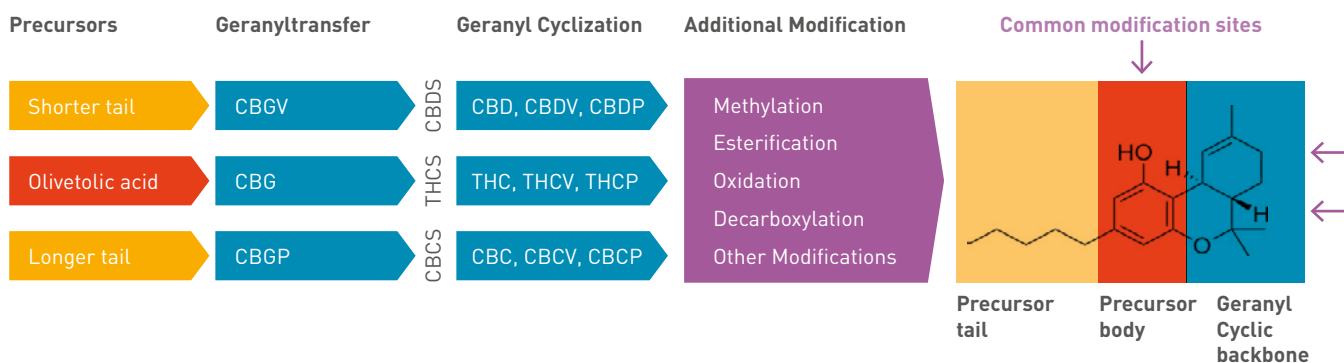
As part of fully understanding how cannabinoids work at the genetic level Dr. Carrera's group is also identifying novel cannabinoids and understanding their biosynthesis and genetic architecture. The genetic diversity of the THC varieties is essential to our work of creating varieties that are enriched or have fixed concentrations of these novel cannabinoids. Moreover, diversity is key for new cannabinoid discovery. To understand cannabinoids a little better we have to appreciate that in the plant cannabinoids like THC, CBD, and CBC exist in an acidic form, denoted THCA, CBDA, and CBCA. When flowers are dried, over time that acid is lost in an event called decarboxylation.

It turns out that THCA, CBDA, and CBCA are all produced on the same assembly line from a precursor called cannabigerolic acid (CBGA). CBGA itself is made from two molecules, olivetolic acid and geranyl pyrophosphate (GPP). In some varieties, small changes in the genome can lead to a substitution of olivetolic acid with a molecule called divarinic acid. This small change creates entirely new cannabinoid diversity, including the varin cannabinoids like tetrahydrocannabivarin (THCV). Different precursors

or different post-cannabinoid synthesis modifications can exponentially make 100s of unique combinations of the products of the three core cannabinoid synthase enzymes THCS, CBDS, and CBCS.

What does this increase in chemical diversity mean for the average cannabis consumer? "There are a lot of extra peaks that we see with our UPLC in this project." —Dr. Carrera notes. Post harvest, Dr. Carrera's committed group analyzed over 10,000 samples, which is orders of magnitude larger than current published research projects. With this large data set they observed rare cannabinoids that are only seen in a few plants. These unknown cannabinoids are generally not stable within the varieties. These unknown peaks can greatly affect user experience. For example, a novel cannabinoid, tetrahydrocannabiphorol (THCP), was recently discovered and it was found to be 30 times stronger than THC. Therefore, even in small undetectable quantities they can greatly change the effects of cannabis on the user. "These unknown cannabinoids are rarely consistent in varieties where they are observed, and it would fit with the hypothesis that the inconsistent affects that consumers experience with cannabis is due to some of the more potent cannabinoids that this plant makes" —Dr. Carrera comments.

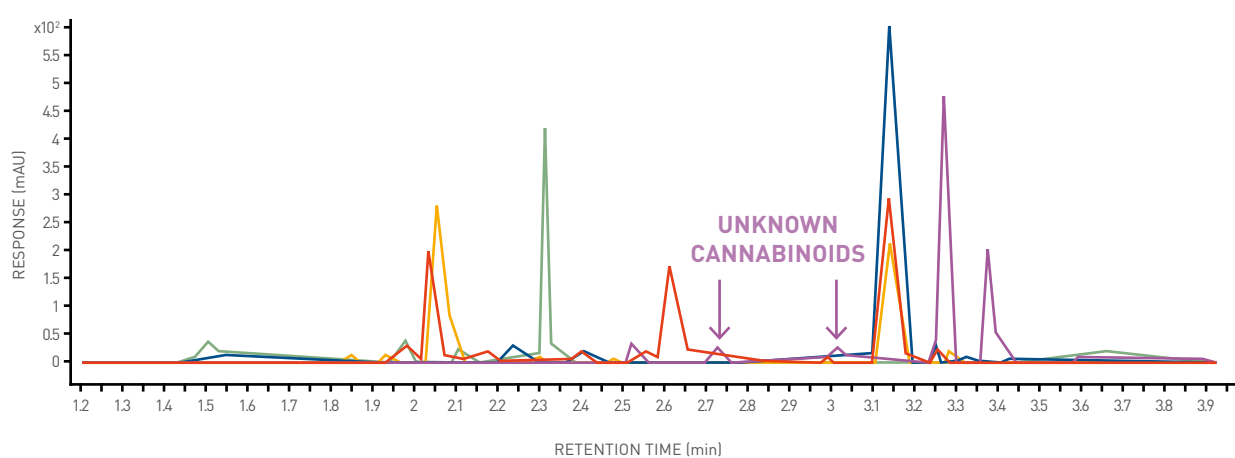
This is just one example of the level of diversity we have seen in our THC field. Having access to THC varieties allows us to make discoveries that were previously impossible because cannabinoids like THCV are connected to the presence of THC. Dr. Carrera says, "What I'm most excited about from our first THC field experiment is the 18 potential unknown cannabinoids that we are now in the process of identifying. They may be breakdown products of other cannabinoids but some of them could be something completely new. We love discovery and having access to the THC varieties strengthens our continuing investigation into the vast chemical diversity of cannabis."



How does cannabis make so many different compounds? Realistically cannabis only has a few unique enzymes in the plant kingdom, the cannabinoid synthases. These enzymes are CBG-synthase, THC-synthase, CBD-synthase, and CBC-synthase. Other enzymes, such as esterases, that modify cannabinoids are not unique to cannabis, but bring the chemical diversity of the plant kingdom and build on the cannabinoid chemical backbone.



CHEMICAL DIVERSITY: THE CHALLENGE OF UNKNOWN COMPOUNDS IN UNDEFINED VARIETIES



- THC dominant
- CBC dominant
- THC:CBD 1:1
- CBG dominant
- Varin dominant

How are cannabinoid measured? At Puregene we use Ultra-high Performance Liquid Chromatography (UPLC). It's faster and more accurate than most methods on the market.



TERPENES PAINTING WITH FLAVOR AND EFFECT

If you have ever wondered where the distinct and pungent smell of cannabis comes from, look in part to the terpenes. This important family of molecules are one of the primary aroma compounds in plants. Just think about the smell of citrus or the gentle smell of lavender and you are on the right track, these come from terpenes. There are over 100 known terpenes in cannabis, but a few are more striking than others giving cannabis flowers their characteristics odors; limonene will lend a citrus fragrance to the flowers; pinene, a fresh pine fragrance; and myrcene can give the flowers an earthy sweet pungent aroma. However, it is the complex mixture of different terpenes in combination with other volatile plant metabolites that ultimately creates the unique aroma and flavor profiles of cannabis flowers.

When the plants in the THC-trial started flowering we were completely stunned by the smell of the field. Actually, it wasn't just the field, almost every plant seemed to have its own unique aroma.

The diversity of individual aromas from the THC-field was by far greater than what we experienced from our CBD-varieties. Finding this incredible array of different flavors supports our hunch that the genetic diversity of the THC-field was rich. Terpene flavors are valued by recreational consumers because they add pleasant and appealing flavors to flowers. But terpenes may also be medically important and current research is decoding an entourage effect where certain terpenes can act with cannabinoids to enhance their effects greatly and specifically.

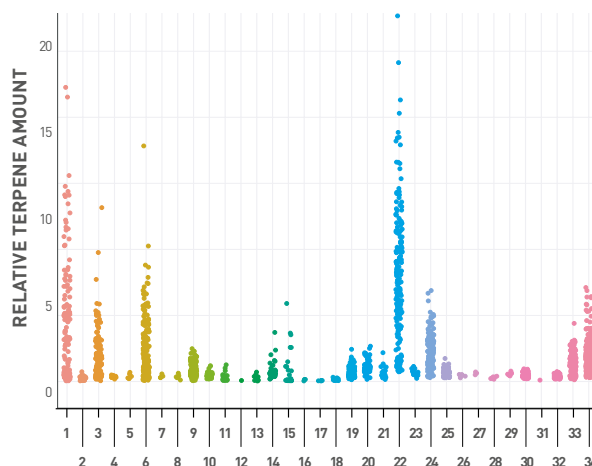
Julian Koschmieder, Head of Analytics at Puregene is also fascinated by terpenes: „Remarkably they have both flavor and activity, that's very rare in chemistry". Julian is leading efforts at Puregene to understand the terpene code. Each cannabis variety has a unique terpene composition, differing in amount and kind. This gets complex when you imagine all the potential combinations. To break this code, he uses gas chromatography to detect the presence of terpenes in diverse cannabis varieties. „We specifically look at the 50 most common terpenes in cannabis. The differences we find between varieties are significant enough for us to begin to identify, through association, the genetic component that encodes a variety's terpene profile."

"Our goal is to use our knowledge of the genetic basis for the terpene code to create varieties in our breeding program with tailored flavor and effect for our customers."

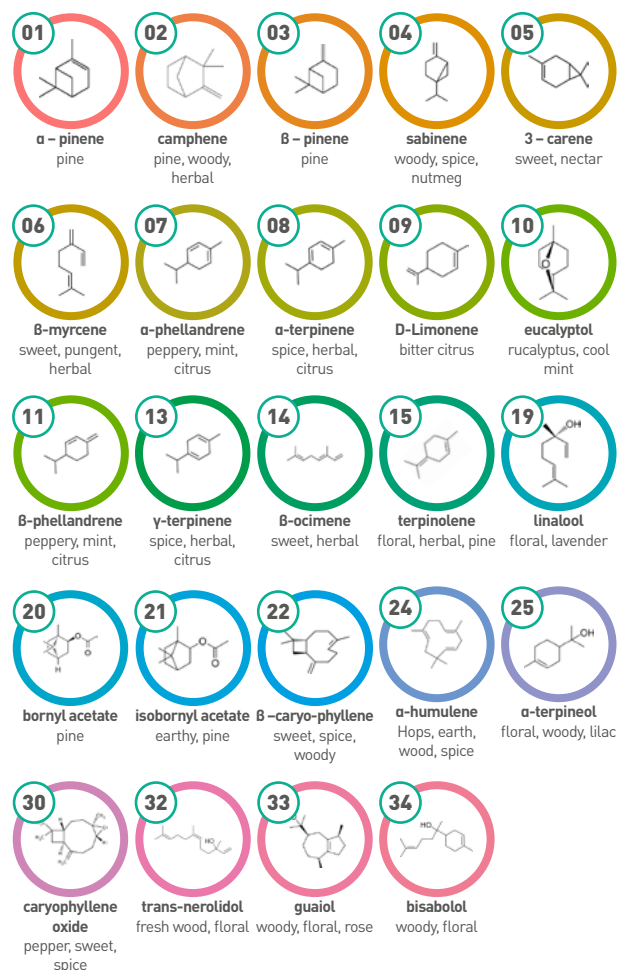
Julian also points out, „It's not only about traits we want to have, there are flavors from terpenes, like those that are a little oniony, some may want to avoid. What's also very exciting is the potential to discover novel terpenes in cannabis. We are just at the start but there are hints that we have found something new."

Fun Fact:

We not only measure terpene profiles on GC but also test for smell the old fashion way. Flavors of passion fruit, pineapple, citrus, wild berries are fairly common, but we also find other stranger smells like onion, grilled beef, wet dog and cucumber.



Which terpene is the most common? 34 different terpenes were observed to detectable levels in the field. β -caryophyllene was the most common and most abundant in most varieties. Myrcene, pinene, humulene, limonene, guaiol, and bisabolol were also common and abundant. The relative abundance between each plant varied greatly, for example β -caryophyllene varied almost 100 fold between the highest and lowest plant. The same diversity was observed for most terpenes. Additionally, there is a strong genetic component that determines this large diversity.





FLOWER QUALITY ON THE FROM THE FIELD: HIT AND MISS

Current black market cannabis is grown indoors in perfect growing conditions with perfectly controlled environments, ample nutrient supply, and uncontrolled levels of pesticides. The flowers grow large with compact calyxes, are light green and full of trichomes, and free of imperfections from diseases. Much like an apple at the store, consumers shop with their eyes. This will be a challenge for growing cannabis in Switzerland, because cold can stop growth and causes the flower to be less compact and more leafy. Diseases are more prevalent and leave spots on the flowers. Trichomes can become damaged by the rain and wind. All factors lower flower quality.

At the end of October 2020 our THC-trial field was in full bloom. Max Vogt Head of Plant Breeding recalls "Some flowers were enormous, compact, pungent, shimmered with trichome, and colorful. The diversity is on a different level than what we see in our CBD varieties. The problem is rarely did we see all of these traits in one plant. A majority of the flowers were barely A grade, and when the market demands AAA grade flowers, less than 10% of the varieties tested could meet that standard." Our team believes that much of the diversity and many of the important agronomic traits we find in the THC varieties are a direct result of intensive breeding that CBD varieties didn't have, so there are many great traits to work with. Many of the most desirable traits for indoor growing have been selected because of their appeal to consumers but this may actually wind up being a disadvantage in outdoor growing conditions. Flower compactness for example is a desired floral trait but in outdoor growing conditions this may make flowers more susceptible to fungal rot. A large part of the work we are doing is in evaluating traits and varieties but also understanding them in context and applicability to Switzerland and diverse environments.

The large diversity in the THC-field is exciting for Dr. Claudio Cropano who is seeking to better understand the genetic basis of important floral traits - flowering time, flower weight, flower area, flower compactness, flower color, pistil color, trichome density and many more. Claudio sees diversity as the palette from which to paint new varieties, but also as the key to unlocking the genetic signatures behind every trait. By first extensively characterizing and converting floral traits of the distinct varieties in the trial field into data, Puregene is building a digital knowledge base that will drive flower improvement. For example, we found that the top flower of trialed varieties had a range of different weights and densities, in some cases a 10 fold difference from the best and worst varieties. We can use diversity this to select for varieties with desired flower size. But Claudio also uses the difference in flower size to identify genetic markers associated with size. This will lead him to also identify the underlying genes responsible. This approach will give us more control in our breeding program and more understanding of the complex metabolic and developmental pathways that control flowering.





NO MALES ALLOWED

Walking through the bright green sea of cannabis during the THC field trial, Head of Plant Breeding, Max Vogt paused mid sentence, like a hawk noticing something no one else could see; a single male flower growing under a leaf. The small bell-shaped structures emerging just below the fan leaves. Ideally, cannabis is a dioecious plant - there are male and female plants, but some cases, female plants can have male and female flowers. The males had to be removed quickly before they fully matured and released pollen. This was a major headache and a lot of work for Vogt and his team, who understood better than most how important it was to remove these males.

Vogt explains, "there are two major reasons to cull males. The first, seed free cannabinoid rich female flowers are what consumers want. This was exactly what we were evaluating in our field trial. The value of any cannabis harvest is hurt when males are present in the field. Even a little pollen means that all female flowers will be fertilized and produce seeds. In production seeded flowers can't be sold, but in a research experiment, seeds take away energy from making cannabinoids, and destroy the experiment. The second and even more important reason is we need control the pollen produced in field, because it could pollenate neighboring fields, and release THC genetics. Vogt and his team monitored the outside edge of controls plant whose only goal was to capture pollen if it escaped. The diligent team did their work, not a single seed was observed in the outside rows. Imagine pollen from a THC field leaking into a non-THC field. Unknowingly a farmer could wind up growing an illegal crop he thought was THC free".

The presence of males is often addressed by the production of feminized cannabis seed. By inducing a female plant to create male flowers, the pollen from these flowers can then be used to fertilize female flowers of that same plant. This will result in the production of seed that when grown will be primarily female. Unfortunately, commercially available feminized cannabis seeds do not comply with Swiss organic standards.

Instead, Swiss growers need to find a different approach. One solution is using clones from a female plant. This will ensure that each clone, like its „mother“ will be female. A second solution developed at Puregene is the early screening of seedlings for genetic markers of „maleness.“ Because gender is encoded in the DNA of cannabis we can test seedlings for the presence or absence of these identifying genetic markers, finding males early before they even go to the field. Both approaches ensure compliance with Swiss organic standards and solve a large problem for cannabis farmers.

Daniel Carrera the Head of Genomic Research at Puregene explains the science behind our approach - „We use Kompetitive allele specific PCR, KASP for short, to detect single nucleotide differences in the genomes of the plant populations we are screening. This is a very robust biochemical assay, that can be high throughput. We can quickly extract DNA from our samples to do thousands of KASP assays per day.“ Take for example, gender, by using KASP to screen a population of seedlings for the genetic signature of maleness we are able to remove male plants long before they begin to make flowers.

During the trial 17% of all plants in the variety trial were removed because of their production of male flowers, some of these were true males but many were hermaphrodite plants.



True male



Early stage hermaphrodite



Late stage hermaphrodite



CANNABIS DISEASES: IS RESISTANCE FUTILE?

As Cannabis emerges as a globally important economic crop, yield loss caused by fungal, bacterial and viral diseases is becoming a pressing issue. The widespread cultivation of cannabis is exposing this new crop to a host of new biotic threats that move to it from other crop species. Strong phytosanitary measures are critical to ensure cannabis production is not limited by disease.

In our own fields we have started to see the challenges associated with introducing unadopted varieties to new conditions. There is no better example than in comparing the susceptibility to disease of our 2020 THC-field trial to our CBD-field trial. What we observed was that almost every THC-variety trialed was infected to some degree with Septoria and botrytis. Septoria is a pathogenic fungus that causes leaf spot specifically on the leaves of plants. It looks like irregular gray to brown lesions. Symptoms typically start on the older fan leaves and can quickly spread to younger leaves. As the disease develops the leaves will shrivel

up and fall off. Wide-spread infection will hurt plant growth and reduce the size of a crop. The most efficient ways to manage it is to remove infected plant tissue as soon as infection becomes evident. Crop rotation, spacing, mulching and removing stagnant water are good organic approaches to manage this disease.

In the later stages of growth, when the plants were flowering, that's when we really started seeing disease emerge in the field. Fungus on the field can ruin a harvest. Mainly we saw our plants attacked by Botrytus and Septoria. Amazingly some varieties were untouched that were directly next to plant varieties that looked terrible.

MAXIMILIAN VOGT (PHD)

Head Breeder
at Puregene AG

Botrytis (B. Cinerea) is a fungal pathogen that causes devastating damage to more than 1000 species including important crop plants like tomato and strawberry. Botrytis can remain hidden until conditions become ideal then it spreads unrelentingly rotting the plant turning the flowers to black mush. Cannabis growers are often surprised by the rapid spread during flowering. It is not uncommon for an entire crop to be lost.



Septoria (Septoria cannabis) is a pathogenic fungus that causes leaf spot specifically on the leaves of cannabis plants. It looks like irregular gray to brown lesions. Symptoms typically start on the older fan leaves and can quickly spread to younger leaves. As the disease develops the leaves will shrivel up and fall off. Wide-spread infection will hurt plant growth and reduce the size of a crop. The most efficient ways to manage it is to remove any infected plant tissue as soon as infection becomes evident. Crop rotation, spacing, mulching and removing stagnant water are organic approaches to managing this disease.

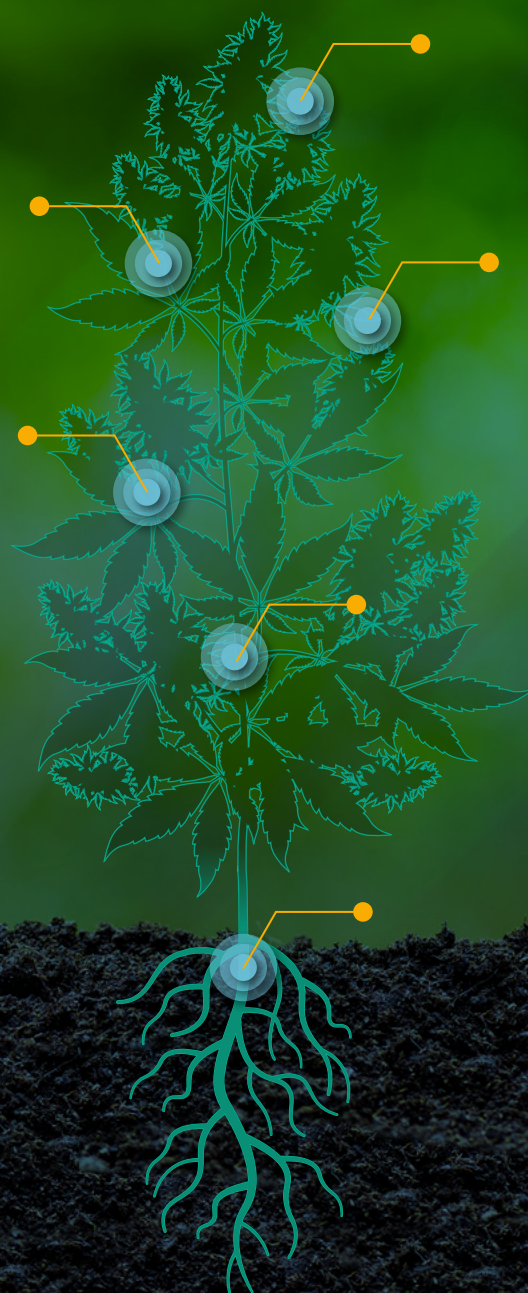




TRAIT DISCOVERY: STEP-ONE TO BUILDING A BETTER CANNABIS PLANT

Trait discovery is central to Puregene's mission of understanding cannabis in order to build the cannabis varieties of the future. To first find traits, we need to observe differences between diverse varieties. The 2020 field trial, and ongoing 2021 trial, represent one of the largest experiments conducted, representing over 1000 THC genotypes and 7000 CBD genotypes. We looked for these differences in three main ways. The first was to carefully observe, measure and digitally record general plant characteristics such as flowering time, height, biomass weight, sex, disease resistance, pistil color, and flower weight. The second evaluated more difficult to evaluate traits at the end of the growing season through image analysis aided by machine learning. This approach gave us access to traits like branching pattern, leaf shape, flower shape, and root architecture. In the third approach the chemical make-up of varieties was evaluated using high throughput analytical chemistry approaches. This determined amount and composition of cannabinoid and terpene. The collection of this trait data becomes a currency of sorts that will drive future investigation and discovery.

Once we focus in on a particular trait, resistance to the fungus Botrytis for example, the next step is to identify the genetic variation or variations that underlie the differences we observe. Genetic variations are small differences in the DNA, these may be at one location or in many depending on the complexity of the trait. Our trait discovery platform brings together trait and genetic variations in an approach called an association studies identifies the genomic regions that associate with a particular trait. This can provide evidence for the role of a particular gene or genes in the inheritance of our traits of interest. Identifying genes and genetic markers that are associated with desired traits is a cornerstone to Puregene's advanced breeding approach. Once the genes are known they can be assembled like bricks to create new plants with unique combinations of traits.



01

CANNABINOIDS

CBD / THC / THCV / THC-free

02

TASTES

Lemon / Strawberry / Spice / Skunk

03

PEST & DISEASE RESISTANCES

Viruses / Botrytis / Mildew / Spider mites

04

YIELD

Faster growth / Taller / Bigger flowers / Bigger Seeds

05

APPEARANCE

Compact flowers / Purple flowers / Branching / Trichomes

06

FLOWERING

No Males / Autoflower / Early-flowering / Fast-flowering

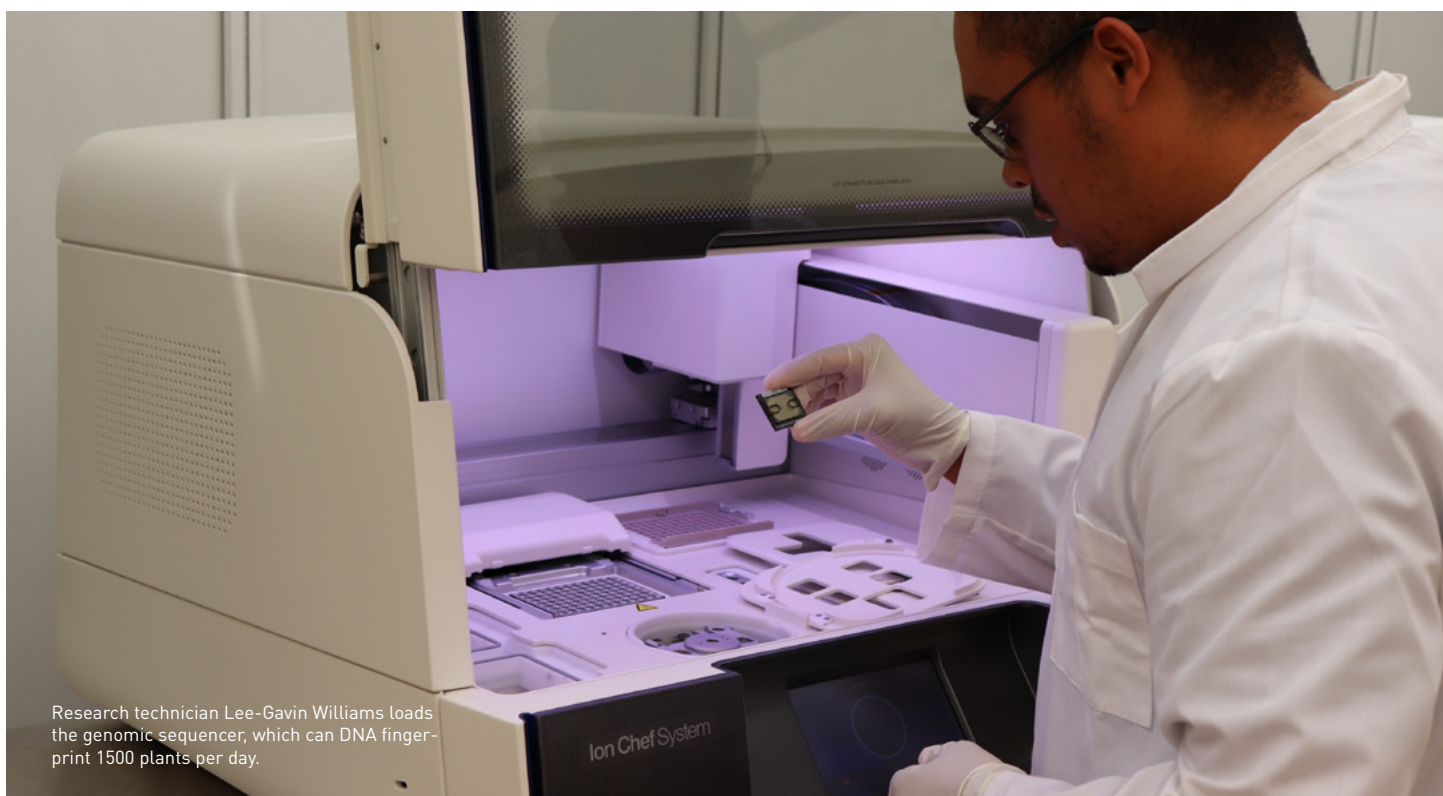
TRAITS



CONCLUSIONS

The 2020 Field Trials for THC and CBD varieties resulted in the collection of an unprecedented volume of detail on cannabis agronomic and chemical traits. The 2020 THC-trial evaluated the performance of over 100 THC-varieties under Swiss outdoor organic conditions as part of a critical step toward supporting the Swiss governments pilot project for THC. Our performance evaluation demonstrated that many of the varieties we tested were genetically unstable and found to have inconsistent THC concentrations amongst siblings. The problem of varietal instability as discussed earlier is a critical problem facing the industry. Until there is a strong regulatory approach to what makes a variety, growers and consumers will be on their own to deal with the variability that come from these poorly defined varieties. This is again the value of conducting a preliminary field trial for

evaluation of varieties. It both brings these critical issues to light but also helped us to identify varieties that are best suited for Switzerland. Still, the issue of variety inconsistency will persist until address. Pure can use technology to bridge the gap until stronger varietal rules are in place. Through the use of genetic fingerprinting, we can ensure varieties are genetically stable and are in fact what they are claimed to be. This can be complemented with chemical analysis to determine the precise cannabinoid concentrations of flowers. We would propose that all varieties in use in Switzerland should be genetically fingerprinted and that flowers going to consumers should be evaluated for their chemical composition. This will not only serve to support the evaluation of the pilot projects but also enrich the general knowledge base connecting variety with chemical composition.



Research technician Lee-Gavin Williams loads the genomic sequencer, which can DNA fingerprint 1500 plants per day.



THE 2021 FIELD TRIAL

The 2021 field trial will continue to carry out experiments for evaluating variety and agronomy parameters for Swiss cultivation of cannabis. It will also be used to validate and further investigate valuable traits identified in the 2020 field trial. Importantly, performance of clones made from varieties will be a central focus of the 2021 field-trials. The use of clones is critical to evaluating the reproducibility of observed traits in effectively identical plants.

Specifically, the research aims of the 2021 experiment will:

Investigate the genetic component of cannabinoid chemistry, sex determination, and photoperiod independent flowering.

Validate key traits identified in the 2020 field trial through the evaluation of 3 clones from each of 347 genotypes.

- Agronomic trait stability and performance of the over 100 traits that were measured in the 2020 experiment will be evaluated.
- The chemical stability and performance testing of 80-100 chemical traits, focused on cannabinoid and terpene profiles, measured in the 2020 experiment will be evaluated.

We will also be introducing the use of plastic foil tunnels to improve flower quality. Foil tunnels help retain heat, protect from weather, and buffer against sudden changes in temperature. Varieties will be compared for performance with tunnels and without.

Finally, an important goal of the 2021 experiment will be to test variety performances or VCU and Variety Distinctness, Uniformity, and Stability (DUS). To test, 6 clones from 39 genotypes will be grown and evaluated in the 2021 experiment. Agronomic and chemical traits will be measured and assessed for each plant as it was in the 2020 field trial.



The 2021 THC-field report is currently being prepared!



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