Cannabis Breeding Program

PSS 4321

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Cannabis Improvement Program

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I have chosen the species Cannabis indica *Lam.* (13), more commonly referred to as Indian hemp or marijuana. Cannabis indica has a diploid genome with twenty chromosomes (11). The traits which I have selected for in my improvement program are; non-photo dependence, a simply inherited trait (7), and a balanced ratio of Cannabidiol and Delta-9 Tetrahydrocannabidiol in the inflorescence of the plant, a quantitative trait (10).

The importance of the quantitative trait which I have selected to work on in my improvement program is due to the large return on high concentration CBD/THC plants in the market place (8), which contributes to the financial returns of a costly breeding program such as the one I have utilized, while simultaneously improving Cannabis indica's ability to be used medicinally for it Cannabidiol content (14).

The importance of the simply inherited trait which I have selected to work on in my improvement program is due to the increased outdoor cultivation area offered by non-photo dependent cannabis crops as well as the cost reducing benefits afforded by the traits ability to circumvent a portion of the immense costs related to indoor, high-intensity, timed and flowering specific lighting that is required for commercial marijuana production (6). My selection of traits accomplishes this by eliminating the need for special lighting and equipment necessary to induce simultaneous flowering of the crop, as used in conventional commercial cannabis cultivation.

The genes targeted in my selection for increased production of delta 9 Tetrahydrocannabidiol in the inflorescence of the plant are a series of three unigenes referred to

as Unigene(s) CAN 4, CAN 7, and CAN 5 (1). Out of all present in the study 72 of 138 EST encoded proteins were CAN 4, 7 and 5 (1). Out of these unigenes the NCBI accessions which had the largest number of expressed sequence tags were ABM21763.1, a Metallothionein-like protein, BAB60848.1 and RD22-like BURP domain-containing protein, and AAL30422.1, a Hevein-like protein, all three of which function as biotic or abiotic stress response proteins in the plant (1). This gene group dictates biosynthesis of CBD and other cannabidiols in the cannabis plant by playing a role in the expression of genes utilized to create secondary metabolites which aid in the production of CBD and THC. These secondary metabolites, such as THCA, which has greater expression in the glands of pistillate inflorescence (1) are concentrated at 400X higher in the inflorescence than the amount found in leaves, leading scientists to conclude that metabolites like THCA are what biochemically produce cannabidiols like THC and CBD (1).

The economic impact of increasing the content of delta-9 Tetrahydrocannabidiol in Cannabis indica would be, and thus far has been, immense. Many strains have been hybridized to gradually increase CBD content in the otherwise heavily THC laden landrace Cannabis indica varieties (4). These hybrids are the most commonly consumed medicinal and recreational cannabis products currently sold in The United States (8). Colorado alone expects to take in 184 million dollars in tax revenue by June 30th, 2015, the end of the next fiscal year (8); most of this tax revenue will come from preparations containing Cannabis indica containing relatively high levels of THC alongside comparable levels of CBD and the unigene groups responsible for their production (2). Strains such as Purple Kush which are prized for their intoxicating effects (2) can

be bred to increase CBD and THC levels to a point where they can equalize in a close to 1:1 ratio, this is seen as an improvement overall as it aids in the reduction of pain symptoms due to the THC, but also serves as a potential treatment of epilepsy due to the levels of CBD.

The genes that impact the traits targeted in my selection for non-photo dependence are those in control of photosynthesis. Genes which affect the enzyme Rubiso, which aides in CO2 fixation, these rates of fixing highly affect the rate of photosynthesis experienced by the plant. The alleles which causes cannabis to be non-photo dependent are recessive (7). Biochemically non-photo dependent Cannabis work by bypassing the vegetative growth typically seen in both Cannabis indica and sativa. The Cannabis ruderalis plant flowers according to age rather than latitude and changes in sunlight, the age of the plant can be determined in degree days and the number of days it has grown since seeding (5).

The economic impact of crossing Cannabis indica and ruderalis would be large. Producers of such strains would not be confined keeping plants in the flowering stage isolated from those in the vegetative stage (7). The impact of non-photo dependent cannabis would be most important in the recreational consumer market, the lowering of operating costs incurred due to expensive indoor lighting would be curbed (6), leading to an overall increase in the per kilogram profit made by the producer. This cross breeding of high CBD/THC C. indica plants with auto flowering C. ruderalis plants would be of special importance to consumers who have little to no skill growing plants indoors, but still seek to grow Cannabis as a hobby. Consumers

who purchase non photo dependent cannabis will have a much easier time understanding how to grow this variety when compared to the difficulty of more traditional C. indica strains. The outdoor cultivation of cannabis used for medicine and recreation would also be able to expand, as non-photo dependent cannabis can grow at higher latitudes than its more equatorial counterparts (5).

To begin my plant breeding program I start with a male German fiber landrace C. ruderalis plant which is homozygous recessive for non-photo dependence (dd). I start by taking this plant as the parent plant and crossing it with my female south Indian marijuana landrace C. indica plant which has a homozygous dominant allele for photo dependence (DD) (9). The cross yields a 100% homozygous population for the F1 generation. This would take place indoors.

Moving on to the F2 generation I self the F1 population resulting in a yield with a 1:2:1 ratio (10) of 25% DD, 50% Dd, and 25%dd. I would then select from this population via light restriction, therefor this generation would take place indoors as well. To obtain my F3 population I would not give the plants proper lighting to induce flowering in photo dependent varieties causing the phenotypic selection of all non-photo dependent plants, the 25% dd as my F3 population.

I would then take my F3 population and begin tissue culture of clones off of the most phenotypically pleasing female plant; these clones would then be grown indoors and selected via transgressive segregation wherein only plants possessing high CBD/THC levels would be

allowed to live onto become my F4 generation. I would then take my F4 generation and continue the process of genetic advance indoors by selecting plants which exhibited higher than average levels of both Cannabidiol and delta-9 Tetrahydrocannabidiol. Those plants would be selected to become my F5 generation.

At my F5 generation (2.5 years into the breeding process) I would continue the genetic advance under selection indoors to push the levels of CBD and THC to greater concentrations in the inflorescence. I would select the plants with the greatest concentration of these chemicals and eliminate the rest leading the way to my F6 generation which will be the last generation of genetic advance under selection for CBD/THC concentration.

Once my program had reached the F6 generation I would begin the final round of transgressive segregation by selecting plants which exceed the average concentration of CBD and THC in the overall population when grown indoors. I would then take these plants and move on to my F7 generation by self-pollination of the virile F6 plants.

After self-pollination the F7 generation would then be separated into four randomly selected groups of plants and moved to four locations which differ primarily in latitude, the soil composition and annual rainfall would have to be as close as possible to ensure that the non-photo dependency of the crop was the main priority. This move to an outdoor scenario would harden the populations and destroy plants only capable of production indoors.

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F8 would be a generation among four different groups of open air pollenated F7 plants. This generation would serve as an opportune time to make phenotypic selection for plants which did not react unfavorably to the change in latitude. Those which struggled to produce levels of CBD/THC such as those in the F7 generation would be disposed of along with plants which showed signs of stunting and reduced yield from change in environment. These four groups of F8 plants would then be open air pollenated amongst their respective groups yet again to form the F9 generation.

In the F9 generation the four groups of plants would undergo yet another selection for hardiness in their new environments, as well as maintenance of stable levels of production of cannabidiols and non-photo dependence. Those plants which exhibited the proper balance of cannabidiols production versus yield would be selected for so long as they were growing properly in their respective latitudes. The plants which met these criteria would go on to become the F10 generation of my cannabis improvement program through open air pollination in their groups.

In the F10 generation the plants would be moved from site 1 to site 2, site 2 to site 3, site 3 to site 4, and site 4 to site 1. This would be to ensure that the plants are adaptable to differing altitudes and climates. All plants would be then open air pollenated and allowed to reproduce creating my F11 generation.

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Once I had my F11 generation established in their new environments I would make a selection based on chemical phenotype looking for a 1:1 ratio, or a close to that as possible, of CBD to THC in the pistillate inflorescence of the plants. The top 25% of plants would be selected from this group and the other 75% would be destroyed. I would then use the remainder to begin another generation founded through open air pollination.

The F12 generation that would emerge would now be suitably adapted to latitude for my liking; therefor they would be moved into greenhouse production yet again for advanced evaluation. In the process of relocating the plants, the top 25% of plants which maintained a chemical phenotype similar to the previous generation, or brought the overall chemotype of the plant closer to a fixed 1:1 ratio of THC to CBD (3) would be selected for and the rest would be discarded.

What remained of the crop would become the end result of my cannabis improvement program. By this point the crop would have become homozygous recessive for non-photo dependence, causing the crop to be classified as an auto flowering variety. The crop would also exhibit higher levels of CBD than other Cannabis indica varieties due to its breeding with Cannabis ruderalis and its extensive process of genetic advance under selection.

The resulting cultivar would be an economic powerhouse for home growers and small scale medicinal production of tinctures due to its cost saving lack of specialty lighting used in production. Another contributing factor that justifies the costly breeding program used to create

this variety is the fact that its overall chemical phenotype, accomplished by combining Cannabidiol and delta-9 Tetrahydrocannabidiol in a closer ratio, would make it an applicable strain for the production of medicinal marijuana products. Products that could potentially focus on both chemical constituents that contribute to the therapeutic benefits of cannabis in the treatment of epilepsy, anxiety, post-traumatic stress disorder, as well as many other numerous ailments.

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Cannabis Improvement Program Flow Chart

"Cannabis ruderalis nonphotodependence X Cannabis indica CBD/THC content"

Two Cannabis individuals, one homozygous non-photodependent C. ruderalis and one homozygous photodependent C.indica, were outcrossed to combine their traits. The resulting population was then bred in order to restore the population to 100% recessive homozygous traits.	
Parents	"German C. ruderalis" dd X "Indian C. indica" DD
F1	4-Dd offspring are created, making the population 100% Heterozygous for photodependence
F2	The F1 generation is then selfed to create a population which segregates at a 1:2:1 ratio, 25% DD, 50% Dd, 25% dd
F3	The F2 population is selected for nonphotodependence by means of light restriction during flowering. The resulting 25% of the population is then back crossed against homozygous nonphotodependent ancestors.
F4	The resulting population contains 100% homozygous recessive nonphotodependent traits. Only 25% of population contains indica traits this 25% is selected by measuring the concentration of CDB and Delta-9 THC in the biomass of the plants.
F5	The plants containing the highest relative amounts of cannabinoids (the 25% containing indica traits) are selected then self polinated.
F6	Transgressive segregation continues as plants are selected for those plants exhibiting the lowest ratio of plant biomass of inflorescense to concentration of cannabinoids in the inflorescence by measuring the weight of the compounds through
F7	Seperation of plants into four different groups to test the effects of change in latitude.
F8-F10	Phenotypic selection of hardy plants. Open air pollenation begins in the field.
F11	Plants are rotated latitudes and open air pollenation continues.
F12	Advanced chemical evaluation of CBD to THC ratio.
RELEASE	

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