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By llightle at 1:30 pm, Jan 18, 2024

Ms. Bernadette Juarez
APHIS Deputy Administrator
Biotechnology Regulatory Services
4700 River Road Unit 147
Riverdale, MD 20737-1236

RE: Regulatory Status Review submission for a new gene edited Cannabis sativa product, Badger G

Submitted by: The Wisconsin Crop Innovation Center of the University of WI-Madison, 8520 University Green, Middleton, WI 53562

Michael Petersen, Senior Scientist, **mwpetersen@wisc.edu**

This RSR request does not contain CBI.

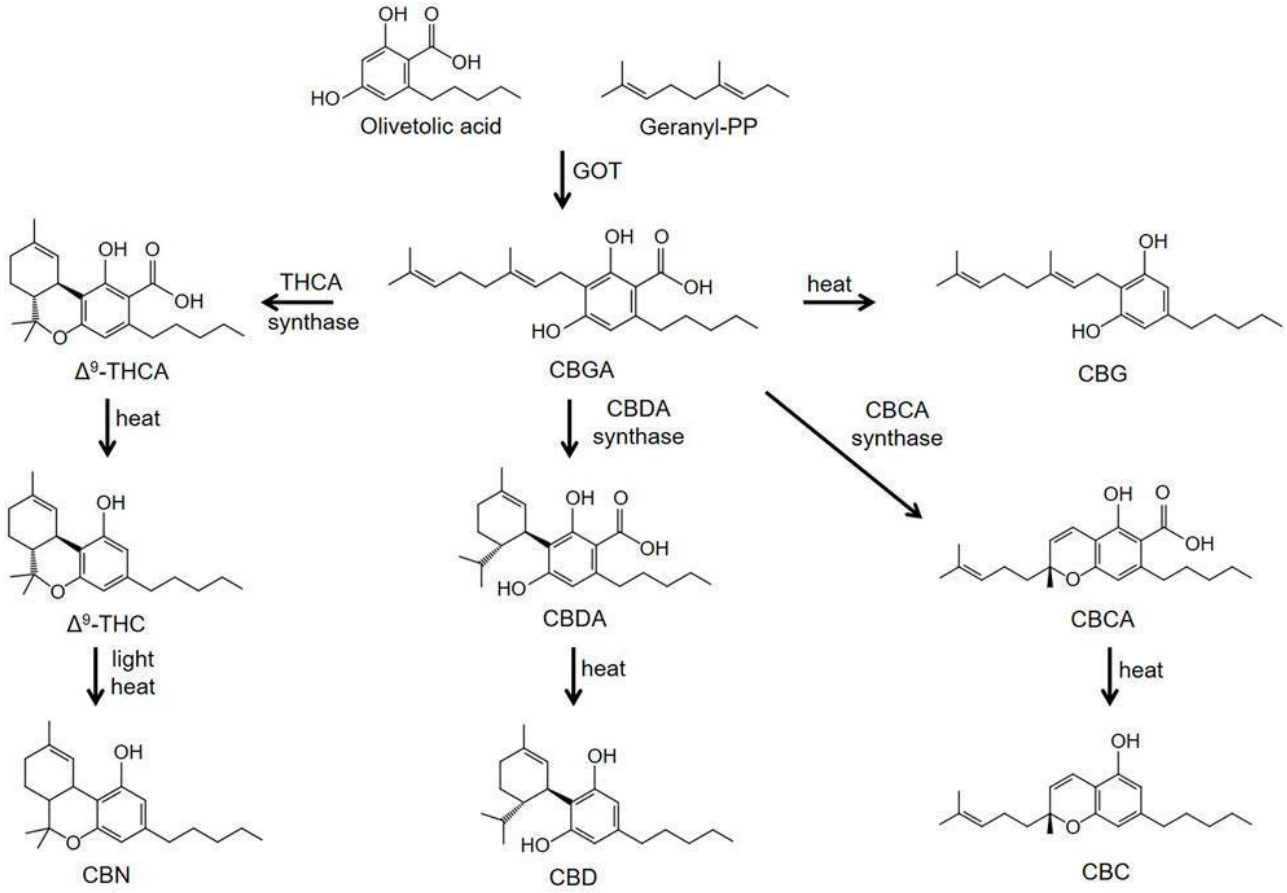
Introduction:

The University of WI-Madison is submitting this document to USDA-APHIS Biotechnology Regulatory Services for a Regulatory Status Review as described in 7 CFR part 340.4. We are requesting a determination of non-regulated status of our new gene edited Cannabis sativa product, Badger G, and any derivatives (progeny) arising from crossing Badger G to other conventional or non-regulated genetically engineered or edited cannabis lines that are not subject to 7 CFR Part 340 regulations. The University of WI-Madison has developed a cannabis plant, Badger G, that is absent of CBD/CBDA and THC/THCA through a gene editing knockout of the endogenous CBDAS gene. This will provide U.S. growers with agronomic and compliance benefits, including higher levels of the cannabinoid CBG/CBGA and elimination of THC/THCA. Approximately 25% of the hemp crop in the US is discarded due to THC/THCA levels beyond the 0.3% threshold set forth in the 2018 Farm Bill. Our new line will allow farmers to be in full compliance with these regulations.

Cannabis sativa Background:

Cannabis sativa L., is grown in many different countries around the world and it has many uses including, food, fiber, oil, medicinal, and recreational. It is generally a diecious plant with separate male and female floral structures on different plants. There are generally three main chemotypes of cannabis: Type 1 – THC dominant; Type 2 – THC+CBD mix; Type 3 – CBD dominant. Due to state and federal regulations, we currently work with Type 3 chemotypes at the University of WI. Figure 1 shows the biosynthetic pathway that produces these cannabinoids.

Fig**. 1:** Illustration of the Cannabinoid Biosynthetic Pathway (Walsh et.al; Front. Pharmacol., 29 November 2021):



Gene Editing Approach to Create Badger G:

It is thought that Type 3 cannabis (hemp) makes small amounts of THC/THCA through its active CBDAS enzyme, since no active THCAS enzymes are usually found in genomes of Type 3 plants. With this in mind, we sought out to knock out the active CBDAS in our Badger genotype using the CRISPR/Cas9 editing approach[1] and designing gRNAs that specifically target the active CBDAS gene. Two gRNAs were used in the original transformation event. Plant transformation was done using a disarmed Agrobacterium rhizogenes strain, 18r12v. A resulting line, Badger G, from the transformation showed a deletion in the CBDAS gene of 487bp. This deletion was directly between the two gRNAs targeting the gene. Upon selfing this line containing the deletion, all the T-DNA inserted sequences were segregated away, leaving only the endogenous CBDAS gene containing the 487bp deletion.

Badger G Molecular Data:

Two gRNAs targeting the active CBDAS of the Badger genotype were designed and included in the original transformation plasmid which also contained the CRISPR/Cas9 elements required for gene editing. PCRs were performed on leaf DNA from Badger G and resulting data showed that a deletion of 487bp was made in the CBDAS gene between

the two gRNAs targets. The PCR band was cloned and sequenced to confirm this deletion within the CBDAS gene in Badger G. Fig. #2 shows the alignment for the deletion of 487bp within the CBDAS gene (Badger3 CBDAS endogenous sequence vs. Badger G with the deletion).

Fig. #2.

Agronomic Characteristics:

The University has grown both T0 and T1 progeny from the Badger G in its greenhouses and it exhibits normal growth to that of the parent. We have identified lines both with and without the CBDAS deletion as well as lines with and without the inserted T-DNA components and all grow similarly.

Conclusion:

The University of WI-Madison has created a unique hemp line, Badger G, using CRISPR/Cas9 gene editing methodology and has not noted any phenotypic changes in this that would indicate any plausible path to increased plant pest risk compared to other hemp varieties. We have identified a T1 progeny that contains a 487bp deletion within the endogenous CBDAS gene, causing a lack of function mutant. The originally inserted T-DNA has also been segregated away, leaving no exogenous DNA behind in the plant. As such, The University is requesting that USDA-APHIS-BRS determine that the Badger G hemp line described in this document and any derivatives (progeny) arising from crossing Badger G to other conventional or non-regulated genetically engineered or edited cannabis lines, are not subject to its regulations in 7 CFR part 340. If you have any questions concerning this submission, please feel free to reach out to me.

Respectfully submitted,

Michael Petersen

Michael Petersen
Senior Scientist
WCIC/University of WI-Madison

References:

1. Z. Feng, B. Zhang, W. Ding, X. Liu, D.L. Yang, P. Wei, *et al.* Efficient genome editing in plants using a CRISPR/Cas system. Cell Res, 23 (2013), pp. 1229-1232.