MatMaCorp's *Cannabis sativa* Sex Determination Test

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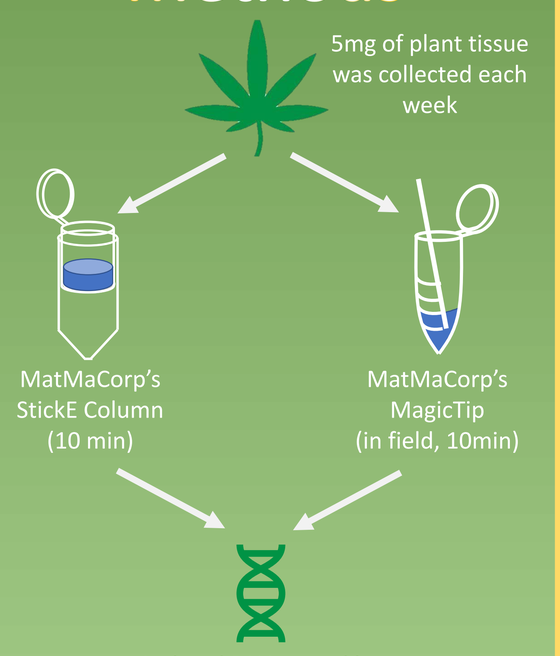
Abstract

Hemp growers can improve their efficiency by increasing the number of female hemp plants available in a field. *Cannabis sativa* (hemp) is a dioecious plant, where female plants flower and produce buds containing high concentrations of CBD compared to their male counterparts. It is vital for hemp growers to identify the sex of each plant as early as possible to isolate female plants and avoid pollination. Early sex identification allows for a greater quality CBD product and saves the hemp grower time and resources that otherwise would have been spent on male plants.

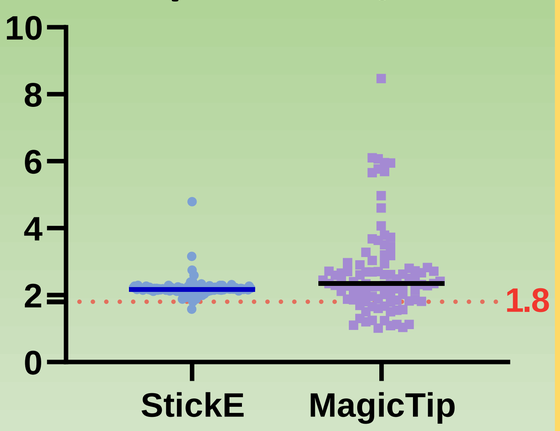
MatMaCorp's *C. sativa* Sexing test allow growers to accurately determine the sex of each hemp plant four weeks after planting which is quicker than determination by PCR or morphology. Chinese long fiber production *C. sativa* seeds were used for this study and were allowed to grow under optimal conditions. MADC6 SCAR marker was used to identify the sex of the plant. Five milligrams of plant tissue were collected weekly after planting. MatMaCorp's StickE column Plant DNA Isolation kit™ and MatMaCorp's MagicTip Plant DNA Isolation kit™ were used to isolate DNA from the plant material. The isolations were then used as template to evaluate MatMaCorp's test.

Methods



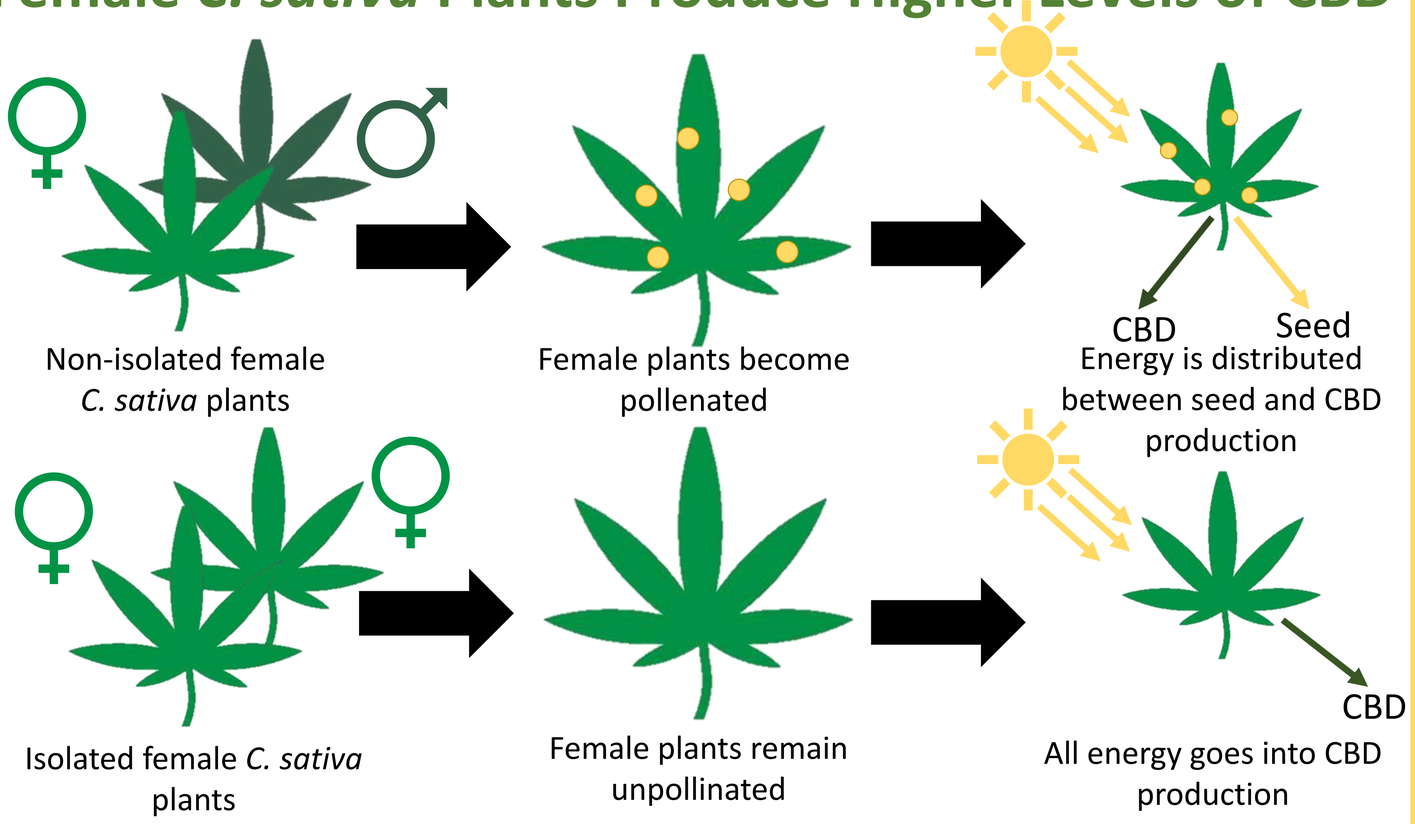
Results

Absorption 260/280

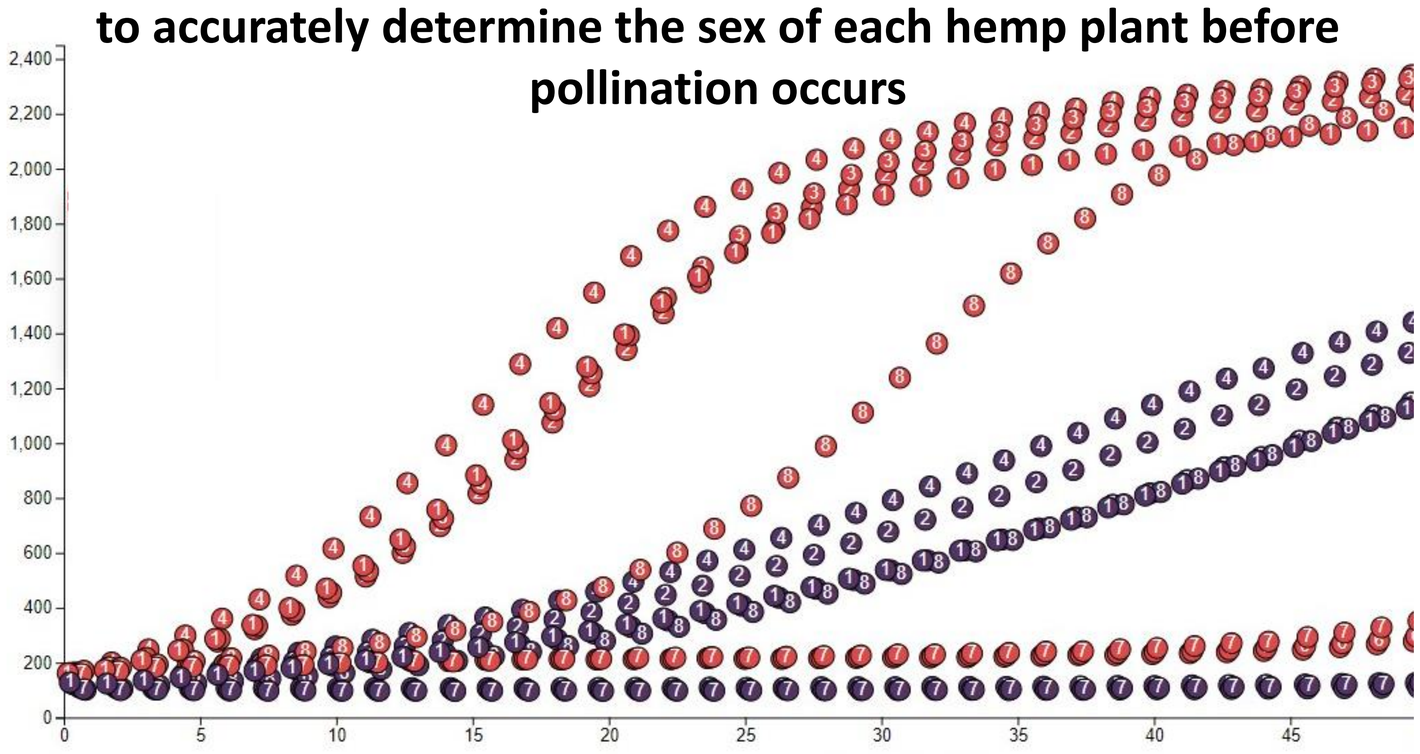


DNA A_{260}/A_{280} ratio comparison between StickE Column and MagicTip (n=184). The ratios were determined by Thermo Scientific NanoDrop™ 2000 spectrophotometer. The red line/ number indicates the optimal ratio for DNA.

Female *C. sativa* Plants Produce Higher Levels of CBD



MatMaCorp's *C. sativa* Sexing C-SAND™ assay allows growers to accurately determine the sex of each hemp plant before pollination occurs



MatMaCorp's *C. sativa* Sexing C-SAND assay developed for identification of the MADC6 SCAR marker. MatMaCorp's StickE Column Plant DNA Isolation kit was used to isolate DNA from two *C. sativa* plants once a week for four weeks. One microliter of isolated DNA was used as template for the C-SAND assay. The isolated DNA used in the C-SAND above is the same DNA used in in the female primer PCR. Therefore, comparisons can be made between C-SAND and PCR.

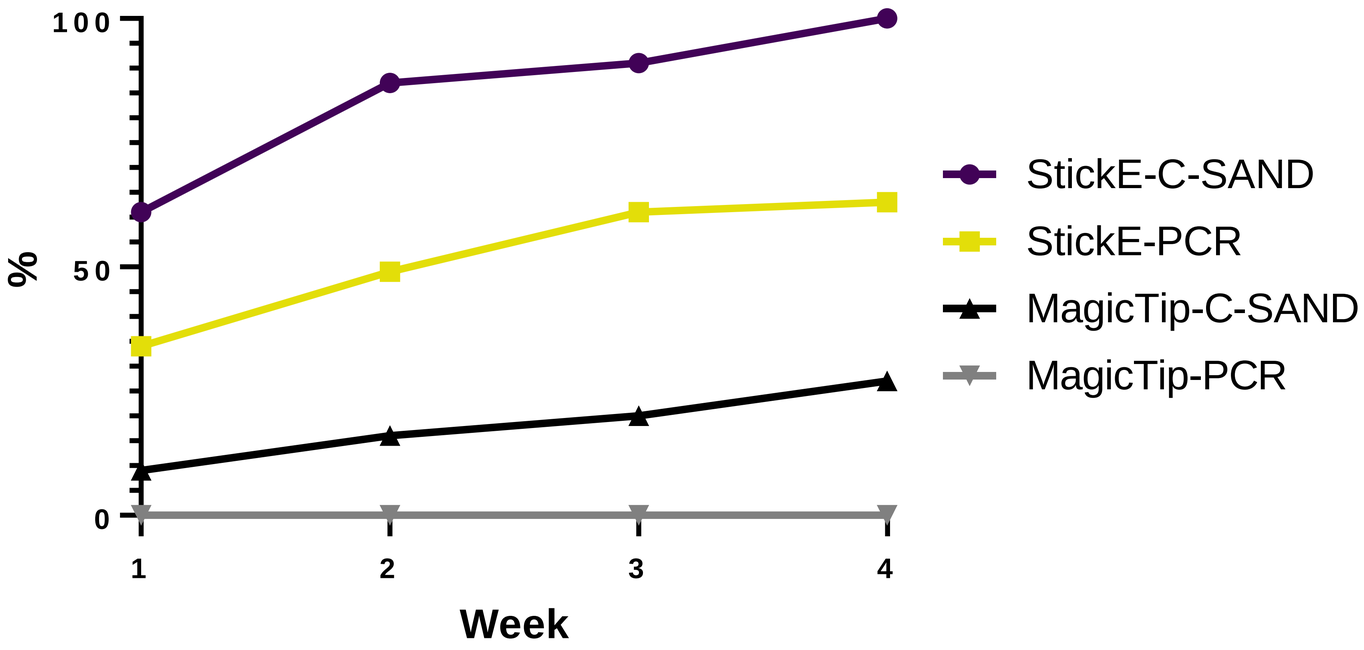
Tube #	Letter	Week
1	A	1
2	A	2
3	A	3
4	A	4
5	B	1
6	B	2
7	B	3
8	B	4

Female

Male

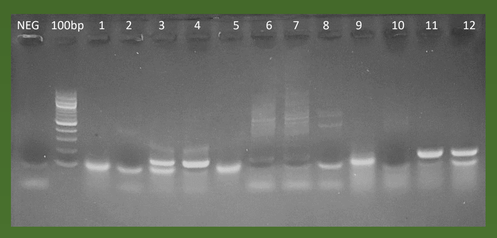
Table 1: Tube # refers to the numbers in red/purple circles shown above. The letter indicates which plant the DNA was isolated from. The week indicates how old the plant was at the time of isolation.

The Percentage of *C. sativa* Plants' Sex Determined per Week

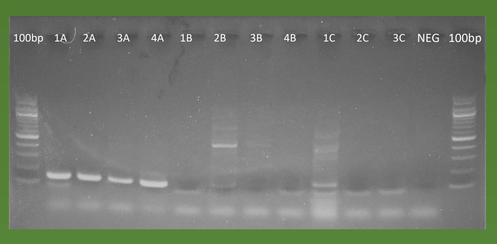


Each week DNA was isolated with both the StickE Column and MagicTip. Both sets of isolated DNA were then used in C-SAND and PCR. The percentages above indicate how many *C. sativa* plants' sex could be determined that week.

PCR

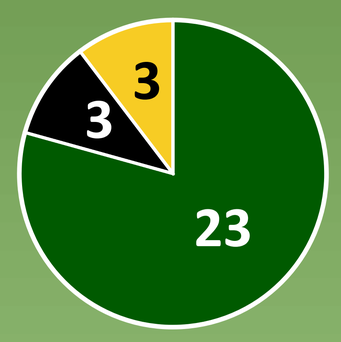


1µL of StickE column isolated DNA from various *C. sativa* plants was used as template for PCR. Male MADC6 SCAR marker primers were used to amplify the region. Product size is approximately 150 bp's.



1µL of DNA from three *C. sativa* plants was isolated using the StickE Column and used as template for PCR. The letters in the gel wells indicate the plant. The numbers in the gel wells indicate the week since the plant had been planted. Female *C. sativa* primers were used. Product size is approximately 150 bp's.

C-SAND vs. PCR Determination



DNA was isolated from 46 four-week-old hemp plants using the StickE Column and tested with PCR and C-SAND. Only 29 plants resulted in a PCR product. Of those products, 26 of the them agreed with C-SAND's sex identification (**Green**). 3 of the products resulted in different identifications. Ex. PCR identifies as male while C-SAND identifies as female (**Black**). **Yellow** indicates C-SAND identified the hemp plant as a hermaphrodite while PCR identified as either male or female not both.

Conclusion

The data from this study suggests *C. sativa* sex can be accurately determined within four weeks by MatMaCorp's *C. sativa* Sexing C-SAND coupled with MatMaCorp's StickE Column Plant DNA Isolation Kit. The combination of these two MatMaCorp's products offer growers:

- Faster sex determination when compared to PCR and morphology
- Sex determination with no need for lab equipment as both products can be used in the field
- Offer higher quality CBD products
- Save time and resources