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Short Communication

Tetrahydrocannabinolic Acid Synthase, the Enzyme Controlling Marijuana Psychoactivity, is Secreted into the Storage Cavity of the Glandular Trichomes

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Tetrahydrocannabinolic acid (THCA) synthase is the enzyme responsible for the production of tetrahydrocannabinol (THC), the psychoactive component of marijuana (*Cannabis sativa* L.). We suggest herein that THCA is biosynthesized in the storage cavity of the glandular trichomes based on the following observations. (i) The exclusive expression of THCA synthase was confirmed in the secretory cells of glandular trichomes by reverse transcription–PCR (RT–PCR) analysis. (ii) THCA synthase activity was detected in the storage cavity content. (iii) Transgenic tobacco expressing THCA synthase fused to green fluorescent protein showed fluorescence in the trichome head corresponding to the storage cavity. These results also showed that secretory cells of the glandular trichomes secrete not only metabolites but also biosynthetic enzyme.

Keywords: Biosynthesis — Glandular trichome — Localization — Marijuana — Tetrahydrocannabinol — Tetrahydrocannabinolic acid synthase.

Abbreviations: CBGA, cannabigerolic acid; GFP, green fluorescent protein; THC, tetrahydrocannabinol; THCA, tetrahydrocannabinolic acid; *THCAS*, tetrahydrocannabinolic acid synthase gene.

Marijuana (*Cannabis sativa* L.) is an annual herb that has been cultivated and used for thousands of years (Fairbairn 1976). It contains unique secondary metabolites called cannabinoids, a group of terpenophenolics. To date, about 70 cannabinoids have been isolated from marijuana (Mechoulam and Ben-Shabat 1999) including Δ^1-tetrahydrocannabinol (THC), a well-known psychoactive component. Besides its psychoactivity, THC possesses analgesic, anti-inflammatory, appetite stimulant and anti-emetic properties, making this cannabinoid a very promising drug for therapeutic purposes (Baker et al. 2003, Guzman 2003).

It is known that cannabinoids such as THC are originally generated in acidic forms. In fresh tissues, the concentration of THC is much lower than that of Δ^1-tetrahydrocannabinolic acid (THCA). THCA is later decarboxylated by a non-enzymatic reaction during storage or smoking (Yamauchi et al. 1967). Despite the long history of marijuana research, the cannabinoid biosynthetic pathway was elucidated only recently. Studies on biosynthetic enzymes over the last decade have revealed the THCA biosynthetic pathway as shown in Fig. 1. Cannabigerolic acid (CBGA), a precursor of THCA, is the product of the alkylation of olivetolic acid with geranyl pyrophosphate by an enzyme called geranylpyrophosphate:olivatolate geranyltransferase (Fellermeier and Zenk 1998). Then, CBGA is converted into THCA by a novel enzyme called THCA synthase (Taura et al. 1995a). THCA synthase catalyzes a unique oxidative cyclization of the geranyl group of CBGA. Recently, we have successfully cloned THCA synthase and characterized its structural and functional properties (Sirikantaramas et al. 2004). Biochemical characterization demonstrated that THCA synthase is a flavinylated oxidase that requires molecular oxygen and produces THCA and hydrogen peroxide. In addition, we have already constructed transgenic tobacco hairy roots expressing THCA synthase that can produce THCA upon feeding of CBGA, suggesting a strategy for the biotechnological production of THC (Sirikantaramas et al. 2004). However, the metabolic engineering of the cannabinoid pathway requires a detailed understanding of the biosynthetic mechanism including regulation and trafficking of the enzymes involved in the pathway. In this study, we describe the cell-specific localization and possible physiological function of THCA synthase, the enzyme responsible for THC production.

With regard to the localization of cannabinoids in *C. sativa*, it has been reported that THC is accumulated only in the secretory cavity of the glandular trichomes (Fujita et al. 1967, Fairbairn 1972). In addition, we have reported that the leaf bud tissue, which is rich in glandular trichomes, contains a potent THCA synthase activity (Taura et al. 1995a). Thus it appears possible that THCA is biosynthesized in the glandular tri-

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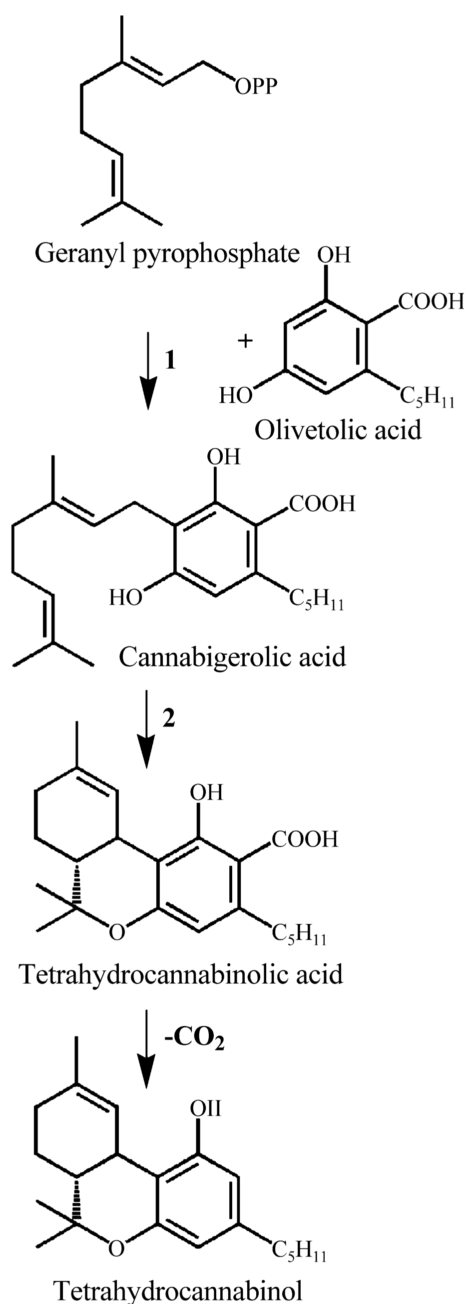

Fig. 1 Biosynthetic pathway of THC. CBGA is a product of the alkylation of olivetolic acid with geranyl pyrophosphate. THCA synthase catalyzes the oxidative cyclization of the monoterpene moiety of CBGA to form THCA. THCA is decarboxylized to THC by a non-enzymatic reaction. The responsible enzymes are: geranylpyrophosphate:olivetolate geranyltransferase (1) and THCA synthase (2).

chomes of *Cannabis* plants. To investigate this possibility, we performed a semi-quantitative reverse transcription–PCR (RT–PCR), using THCA synthase-specific primers, with cDNA samples from the glands and the tissue after gland removal. We used seed coats as a starting material since they provide a large number of glands. As a result, a much larger amount of PCR product, amplified by THCA synthase-specific primers, was detected for the sample from glandular trichomes (Fig. 2). In contrast, only a trace amount of PCR product was detected with the cDNA from gland-removed tissue. The control *18S rRNA* gene fragment was amplified equally for both samples. These results suggested that THCA synthase is expressed exclusively in the glandular trichome.

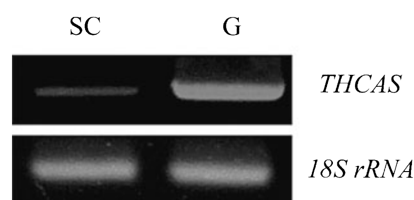

Fig. 2 Semi-quantitative RT–PCR analysis of the expression of THCA synthase gene in the glandular trichome. SC, seed coats after removal of the glandular trichomes; G, glandular trichomes. *18S rRNA* was amplified to verify the amount of template cDNA.

We previously have reported the characteristics of the amino acid sequence of THCA synthase (Sirikantaramas et al. 2004). The sequence contains a 28 amino acid signal peptide and eight putative *N*-glycosylation sites. In addition, a recombinant THCA synthase from an insect cell culture was secreted into the medium, suggesting the trafficking of THCA synthase from the endoplasmic reticulum to the outside of the cell. However, the destination of the protein in plants might be different from that in insect cells. Therefore, we performed a stable transformation to produce transgenic tobacco BY-2 cells harboring *THCAS* to confirm whether THCA synthase is secreted from plant cells. It is noted that we used tobacco as a host because it is difficult to transform *C. sativa* plant stably. Interestingly, enzyme assays with cell extract and culture medium from transformed BY-2 demonstrated that most of the THCA synthase activity (~95%) was secreted into the culture medium. This result suggested that THCA synthase is a secreted biosynthetic enzyme.

The results of reverse transcription–PCR (RT–PCR) and heterologous expression suggest that THCA synthase is localized in the apoplastic space of the glandular trichome. Thus, we next prepared transgenic tobacco plants expressing a green fluorescent protein (GFP)-tagged THCA synthase (THCA synthase–GFP) to visualize the expression of THCA synthase in the glandular trichome. The control GFP-expressing transgenic tobacco cells showed green fluorescence only in the nucleus and cytoplasm. In contrast, GFP-tagged THCA synthase shows green fluorescence all over the trichome head, demonstrating the localization of the fusion protein in the storage cavity (Fig. 3). We also confirmed THCA synthase activity in the leaf tissues containing glandular trichomes, suggesting that THCA synthase–GFP is functionally expressed. Taken together, a sorting pathway for THCA synthase from

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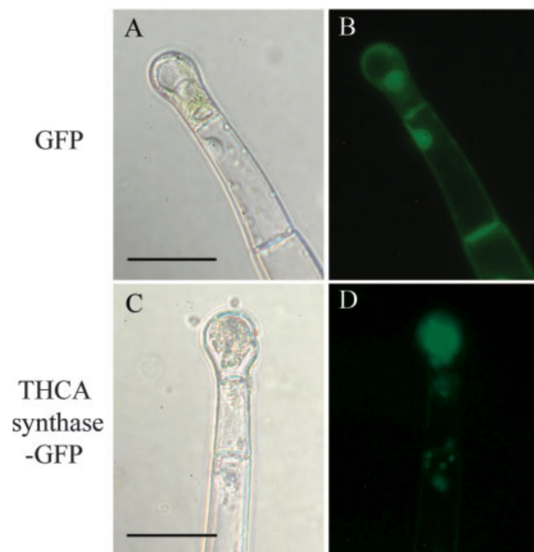

Fig. 3 The localization of THCA synthase–GFP in the storage cavity of the glandular trichomes of transgenic tobacco plants. The expression of control GFP visualized under a phase contrast microscope (A) or fluorescence microscope (B). The expression of THCA synthase–GFP visualized under a phase contrast microscope (C) or fluorescence microscope (D). Bars = 0.05 mm.

secretory cells into the storage cavity is suggested. This finding is interesting since the glandular trichome, which is believed to secrete small molecules such as resinous compounds or volatile oils, can also secrete biosynthetic enzyme into the storage cavity.

To obtain further evidence that THCA synthase is sorted into the storage cavity of the glandular trichome, we directly withdrew the contents of the cavity of *C. sativa* using a stretched glass pipette without destroying the secretory cells, and analyzed the enzyme activity. As expected, THCA synthase activity (~0.01 picokatal) was readily detected for the storage cavity content of individual glandular trichomes. In addition, HPLC analysis showed the presence of CBGA, the substrate of the enzyme, and THCA in the same sample. These results indicated that THCA synthase is actually localized to and functions in the storage cavity of glandular trichomes of *Cannabis* plants. Therefore, the storage cavity is not only the site for the accumulation of cannabinoids but also the site for THCA biosynthesis.

The finding that THCA synthase is secreted into the storage cavity raises an interesting question: why must this enzyme be secreted for the biosynthesis of THCA? In general, secondary metabolites are toxic and must be expelled from the cytoplasm after their biosynthesis. For example, anthocyanin is imported into the vacuole by a specific transporter to prevent cellular damage (Goodman et al. 2004). In the case of cannabinoids, it has been reported that THC is toxic and induces the death of mammalian cells in vitro (Guzman et al. 2001). However, there is no report about other cannabinoids. Since THCA is biosynthesized outside the cell, it is possible that acidic cannabinoids, such as THCA, are also cytotoxic. We, therefore, examined the toxicity of CBGA and THCA, in suspension-cultured *C. sativa* cells and tobacco BY-2 cells, and compared it with that of olivetolic acid, a phenolic moiety in cannabinoids. In 10-day-old suspension-cultured cells of *C. sativa*, 24 h treatment with CBGA and THCA at 50 μM caused 100% cell death as demonstrated by trypan blue staining, whereas olivetolic acid did not have any effect on the cells (Fig. 4A). The same result was observed in 7-day-old tobacco BY-2 cells treated with these two cannabinoids and olivetolic acid. Moreover, we confirmed that CBGA and THCA induce cell death

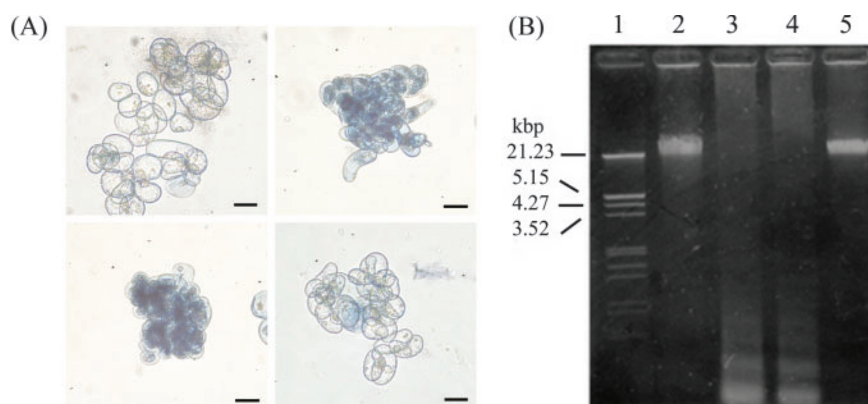

Fig. 4 Effects of cannabinoids on suspension-cultured cells of *C. sativa*. (A) Trypan blue staining of CBGA-, THCA- or olivetolic acid-treated cells of *C. sativa*. Ten-day-old cell cultures were incubated with 50 μM CBGA, THCA or olivetolic acid for 24 h. The cells were stained with trypan blue and visualized under a phase contrast microscope. 1, untreated *C. sativa* cells; 2, CBGA-treated *C. sativa* cells; 3, THCA-treated *C. sativa* cells; and 4, olivetolic acid-treated *C. sativa* cells. Bars = 0.2 μm. (B) Agarose gel electrophoresis of genomic DNA from suspension-cultured cells of *C. sativa*. Genomic DNA was extracted from cell cultures treated with CBGA, THCA or olivetolic acid as described in (A). DNA was separated on a 1% (w/v) agarose gel and detected by ethidium bromide staining. Lane 1, marker DNA with the indicated sizes; lane 2, untreated cells; lane 3, CBGA-treated cells; lane 4, THCA-treated cells; and lane 5, olivetolic acid-treated cells.

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via apoptosis since DNA laddering was detected by gel analysis of genomic DNA from treated plant cells (Fig. 4B). These results clearly showed that cannabinoids are toxic substances. We also confirmed that both CBGA and THCA induced apoptosis not only in plant cells but also in insect (*Spodoptera frugiperda*, Sf9) cells (data not shown), suggesting that cannabinoids act as plant defense compounds, like many secondary metabolites biosynthesized in the glandular trichome. Since cannabinoid-producing glandular trichomes are distributed in physically fragile young tissues of the *Cannabis* plant, THCA and CBGA would protect these tissues from predators such as insects. This is the first report suggesting the physiological importance of THCA and CBGA as apoptosis-inducing defense compounds.

As previously reported, the THCA synthase reaction produces hydrogen peroxide as well as THCA during the oxidation of CBGA (Sirikantaramas et al. 2004). Since the *Cannabis* plant produces a very large amount (~2% of dried tissue) of THCA (Shoyama et al. 1975), a toxic amount of hydrogen peroxide might also be accumulated in the storage cavity of the glandular trichome as a result of the THCA synthase reaction. Thus, THCA synthase might contribute to the self-defense of *Cannabis* plants by producing both THCA and hydrogen peroxide. In addition, since these reaction products are toxic to *C. sativa* itself, THCA synthase must be secreted from secretory cells into the storage cavity to avoid cellular damage.

Cannabis sativa contains various cannabinoids that share a similar structure to THCA. For example, cannabidiolic acid and cannabichromenic acid are isomers of THCA having different ring structures. We have reported that these cannabinoids are biosynthesized from the common substrate CBGA, via a mechanism similar to that of the THCA synthase reaction (Taura et al. 1996, Morimoto et al. 1998). Therefore, these cannabinoids might also be biosynthesized in the storage cavity of the glandular trichome, and may be involved in self-defense in the *Cannabis* plant. It is of interest why *C. sativa* produces such a variety of cannabinoids and whether these cannabinoids have different physiological functions.

Materials and Methods

Suspension cells of *C. sativa* were cultured in a Murashige–Skoog medium containing B5 vitamins (Murashige and Skoog 1962, Gamborg et al. 1968) with 2.5 μM 2,4-D as described by Feeney and Punja (2003). Suspension-cultured tobacco cells (BY-2) were obtained from RIKEN bioresource center (Tsukuba, Japan), and cultured in a modified Murashige–Skoog medium (Nagata et al. 1992). *C. sativa* (Mexican strain) and tobacco (*Nicotiana tabacum* cv. Xanthi) plants were cultured as described previously (Sirikantaramas et al. 2004).

cDNA encoding THCA synthase was cloned into the binary vector pBI121 (Clontech, Palo Alto, CA, USA) (Sirikantaramas et al. 2004). The GFP gene used in this work is the synthetic variant S65T and was inserted between the cauliflower mosaic virus (CaMV) 35S promoter and the polyadenylation signal from the nopaline synthase gene in pUC18 (Niwa et al. 1999).

For construction of the THCA synthase–GFP chimeric gene, primers 5′-TTCAGCGTCGACAACAATGAATTGCTCAG-3′ and 5′-CTAGCTCCATGGAATGATGATGCGGTGGAA-3′ were used to amplify the full-length *THCAS*. The PCR product was digested with *Sal*I and *Nco*I (underlined), and cloned into pUC18–GFP. The complete coding sequence was excised from pUC18 harboring the chimeric gene together with CaMV 35S and the polyadenylation signal by use of *Hin*dIII and *Eco*RI, and recloned into pBI121. All of the constructs in pBI121 as described above were introduced into *Agrobacterium tumefaciens* LBA4404 by tri-parental mating (Van Haute et al. 1983).

Tobacco BY-2 cells were transformed by *Agrobacterium* infection as described (An 1987). For the construction of transgenic tobacco plants, tobacco was transformed using the *Agrobacterium* leaf disc transformation method (Horsch et al. 1985). The transformed tobacco was selected using kanamycin and regenerated as described by Mathis and Hinchee (2000).

The glandular trichomes isolated from 30 seeds and gland-removed seed coats, prepared as described previously (Yerger et al. 1992), were immediately ground in a mortar in the presence of liquid nitrogen. Total RNA was isolated using a Plant Total RNA Extraction Miniprep System (Viogene, Sunnyvale, CA, USA). cDNA was synthesized from total RNA (200 ng) using a random hexamer primer with ReverTra Ace reverse transcriptase (Toyobo Co., Ltd., Osaka, Japan) according to the manufacturer's instructions. PCR using a gene-specific primer pair was performed for 30 cycles (*THCAS*) and 20 cycles (*18S rRNA*) under the following conditions: 94°C for 1 min, 50°C for 1 min and 72°C for 2 min. The 5′ primer and 3′ primer were, for *THCAS*, 5′-CGGGATCCATGAATTGCTCAGCATTT-3′ and 5′-CTAGCTGAGCTCTTAATGATGATGCGGTG-3′, and for *18S rRNA*, 5′-TTCTTGGATTTATGAAAGACGAACAAC-3′ and 5′-AAGACCAACAATTGCAATGATCTATCC-3′.

THCA synthase activity was measured as follows. The enzyme solution (50 μl) was added to the standard reaction mixture consisting of 200 μM CBGA, 0.1% (w/v) Triton X-100 and 100 mM sodium citrate buffer (pH 5.5) in a total volume of 200 μl. The reaction mixture was incubated at 30°C for 2 h. The reaction product, THCA, was detected by HPLC as described previously (Taura et al. 1995a).

A stretched glass pipette was used to collect the secretory products from individual capitate-stalked glands from the seed coat of *C. sativa*. The secretory products were analyzed for cannabinoid content and THCA synthase activity by HPLC (Taura et al. 1995a).

GFP fluorescence in transgenic tobacco was observed under a Nikon TE-2000 microscope (Tokyo, Japan) with a GFP-B filter set (excitation filter, 480 ± 40 nm; barrier filter, 535 ± 50 nm). Images were taken with a Nikon D100 digital camera.

Olivetolic acid was chemically synthesized by carboxylation of olivetol with methyl magnesium carbonate (Mechoulam and Ben-Zvi 1969). CBGA was chemically synthesized by a two-step procedure: condensation of olivetol and geraniol to synthesize cannabigerol (Mechoulam and Yagen 1969), followed by carboxylation of cannabigerol with methyl magnesium carbonate. THCA was purified from dried leaves of *C. sativa* as described previously (Taura et al. 1995b).

Ten-day-old suspension cultured cells of *C. sativa* or 7-day-old tobacco BY-2 cells at a 5-fold dilution were incubated with various concentrations of CBGA, THCA or olivetolic acid (5–50 μM) for 24 h. The viability of the cells was tested by addition of trypan blue solution as described previously (de Pinto et al. 1999). DNA was isolated from *C. sativa* and tobacco BY-2 cells with the CTAB method (Murray and Thompson 1980). Then, electrophoresis was performed in 1.0% (w/v) agarose gels.

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References

An, G. (1987) Binary Ti vectors for plant transformation and promoter analysis. *Methods Enzymol.* 153: 292–305.

Baker, D., Pryce, G., Giovannoni, G. and Thompson, A.J. (2003) The therapeutic potential of cannabis. *Lancet Neurol.* 2: 291–298.

de Pinto, M.C., Francis, D. and De Gara, L. (1999) The redox state of the ascorbate dehydroascorbate pair as a specific sensor of cell division in tobacco BY-2 cells. *Protoplasma* 209: 90–97.

Fairbairn, J.W. (1972) The trichomes and glands of *Cannabis sativa* L. *Bull. Narc.* 23: 29–33.

Fairbairn, J.W. (1976) The pharmacognosy of cannabis. *In* Cannabis and Health. Edited by Graham, J.D. pp. 3–20. Academic Press, London.

Feeney, M.and Punja, Z.K. (2003) Tissue culture and *Agrobacterium*-mediated transformation of hemp (*Cannabis sativa* L.). *In vitro Cell Dev. Biol.* 39: 578–585.

Fellermeier, M. and Zenk, M.H. (1998) Prenylation of olivetolate by a hemp transferase yields cannabigerolic acid, the precursor of tetrahydrocannabinol. *FEBS Lett.* 427: 283–285.

Fujita, M., Hiroko, S., Kuriyama, E. ad Shigehiro, M. (1967) Studies on *Cannabis*. II. Examination of the narcotic and its related components in hemps, crude drugs, and plant organs by gas–liquid chromatography and thin-layer chromatography. *Annu. Rep. Tokyo Coll. Pharm.* 17: 238–242.

Gamborg, O.L., Miller, R.A. and Ojima, K. (1968) Nutrient requirements of suspension cultures of soybean root cells. *Exp. Cell Res.* 50: 151–158.

Goodman, C.D., Casati P. and Walbot, V. (2004) A multidrug resistance-associated protein involved in anthocyanin transport in *Zea mays*. *Plant Cell.* 16: 1812–1826.

Guzman, M. (2003) Cannabinoids: potential anticancer agents. *Nat. Rev. Cancer* 3: 745–755.

Guzman, M., Sanchez, C.and Galve-Roperh, I. (2001) Control of the cell survival/death decision by cannabinoids. *J. Mol. Med.* 78: 613–625.

Horsch, R.B., Fry, J.E., Hoffmann, N.L., Eichholtz, D., Rogers, S.G. and Fraley, R.T. (1985) A simple and general-method for transferring genes into plants. *Science* 227: 1229–1231.

Mathis, N.L. and Hinchee, M.A.W. (2000) *Agrobacterium* inoculation techniques for plant tissues. In *Plant Molecular Biology Manual*. Edited by Gelvin, S.B. and Schilperoort, R.A. B6: pp. 1–9. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Mechoulam, R. and Ben-Shabat, S. (1999) From gan-zi-gun-nu to anandamide and 2-arachidonoylglycerol: the ongoing story of cannabis. *Nat. Prod. Rep.* 16: 131–143.

Mechoulam, R. and Ben-Zvi, Z. (1969) Carboxylation of resorcinols with methylmagnesium carbonate. Synthesis of cannabinoid acids. *J. Chem. Soc. D* 1969: 343–344.

Mechoulam, R. and Yagen, B. (1969) Stereoselective cyclizations of cannabinoid 1,5 dienes. *Tetrahedron Lett.* 60: 5349–5352.

Morimoto, S., Komatsu, K., Taura, F. and Shoyama, Y (1998) Purification and characterization of cannabichromenic acid synthase from *Cannabis sativa*. *Phytochemistry* 49: 1525–1529.

Murashige, T. and Skoog, F. (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15: 473–497.

Murray, M.G. and Thompson, W.F. (1980) Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res.* 8: 4321–4325.

Nagata, T., Nemoto, Y. and Hasezawa, S. (1992) Tobacco BY-2 cells as the “Hela” cell line in the cell biology of higher plants. *Int. Rev. Cytol.* 132: 1–30.

Niwa, Y., Hirano, T., Yoshimoto, K., Shimizu, M. and Kobayashi, H. (1999) Non-invasive quantitative detection and applications of non-toxic, S65T-type green fluorescent protein in living plants. *Plant J.* 18: 455–463.

Shoyama, Y., Yagi, M., Nishioka, I. and Yamauchi, T. (1975) Biosynthesis of cannabinoid acids. *Phytochemistry* 14: 2189–2192.

Sirikantaramas, S., Morimoto, S., Shoyama, Y., Ishikawa, Y., Wada, Y. and Taura, F. (2004) The gene controlling marijuana psychoactivity: molecular cloning and heterologous expression of Δ^1-tetrahydrocannabinolic acid synthase from *Cannabis sativa* L. *J. Biol. Chem.* 279: 39767–39774.

Taura, F., Morimoto, S. and Shoyama, Y. (1995b) Cannabinerolic acid, a cannabinoid from *Cannabis sativa*. *Phytochemistry* 39: 457–458.

Taura, F., Morimoto, S. and Shoyama, Y. (1996) Purification and characterization of cannabidiolic-acid synthase from *Cannabis sativa* L. Biochemical analysis of a novel enzyme that catalyzes the oxidocyclization of cannabigerolic acid to cannabidiolic acid. *J. Biol. Chem.* 271: 17411–17416.

Taura, F., Morimoto, S., Shoyama, Y. and Mechoulam, R. (1995a) First direct evidence for the mechanism of Δ^1-tetrahydrocannabinolic acid biosynthesis. *J. Amer. Chem. Soc.* 117: 9766–9767.

Van Haute, E., Joos, H., Maes, M., Warren, G., Van Montagu, M. and Schell, J. (1983) Intergeneric transfer and exchange recombination of restriction fragments cloned in pBR322: a novel strategy for the reversed genetics of the Ti plasmids of *Agrobacterium tumefaciens*. *EMBO J.* 2: 411–417.

Yamauchi, T., Shoyama, Y., Aramaki, H., Azuma, T. and Nishioka, I. (1967) Tetrahydrocannabinolic acid, a genuine substance of tetrahydrocannabinol. *Chem. Pharm. Bull. (Tokyo)* 15: 1075–1076.

Yerger, E.H., Grazzini, R.A., Hesk, D., Cox-Foster, D.L., Craig, R. and Mumma, R.O. (1992) A rapid method for isolating glandular trichomes. *Plant Physiol.* 99: 1–7.

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