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Growth characteristics of *Cannabis sativa* L. cultivated in a phytotron and in the field

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Abstract

Growth characteristics of *Cannabis sativa* L. are indispensable factors to verify the statements by the criminals of illegal cannabis cultivation. To investigate growth characteristics of *C. sativa*, two varieties, cannabidiolic acid (CBDA)-rich (CBDA-type) which being cultivated for fiber production and △^{9}-tetrahydrocannabinolic acid (THCA)-rich (THCA-type) which is used for drug abuse, were cultivated from seeds under the same growth environment in a phytotron. THCA-type showed high germination rate (100%) whereas only 39% of the CBDA-type seeds germinated 6 days after sowing. Plant height, number of true leaves, number of nodes, number of axillary buds and flowering of these two varieties were periodically observed. THCA-type grew more rapidly (plant height: 125.8 cm for THCA-type, 84.7 cm for CBDA-type, 75 days after cultivation) demonstrating vigorous axillary bud formation and earlier male-flowering (63 days for THCA-type, 106 days for CBDA-type, after sowing). Propagation of THCA-type was tested using the axillary shoot cuttings of female plants either with or without the main stem. All the cuttings with the main stem rooted after 21 days and grew healthily in a phytotron. However, all the newly developed leaves were single instead of palmate. In the field, THCA-type male-flowered after 155 days of cultivation after sowing on March 31. The height of the field-cultivated plants reached 260.9 cm 163 days after sowing. Despite the great differences in final plant heights, the increases of plant height per day during the vegetative growth stage were similar in the field and in the phytotron. Thus estimating the starting time of illegal cannabis cultivation might be possible if the plant is in the vegetative growth stage.

Keywords: *Cannabis sativa*, Cannabaceae, THCA, CBDA, cuttings

Introduction

Cannabis plant, *Cannabis sativa* L. (Family: Cannabaceae), is dioecious and annual. The plant is used in a variety of ways e.g. as furnishing fiber, oil, medicine, and as narcotics[1)]. A class of secondary metabolites unique to this plant is terpenophenolics known as cannabinoids (Fig. 1), which accumulate mainly in the glandular trichomes of the plant[1, 2)]. Among the cannabinoids isolated so far, the most abundant ones are cannabidiol (CBD) and △^{9}-tetrahydrocannabinol (THC), and the latter has strong psychoactive property. In cannabis plants, cannabinoids are synthesized and accumulated as cannabinoid acids such as cannabidiolic acid (CBDA) and △^{9}-tetrahydrocannabinolic acid (THCA). When the herbal product is dried, stored, or heated, the acids are decarboxylated partly or completely to give their neutral forms (e.g., CBDA into CBD, THCA into THC)[2)].

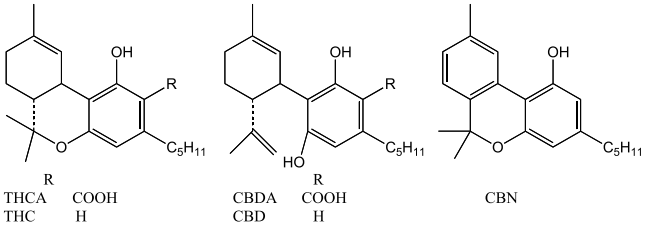

Fig. 1 Cannabinoids in Cannabis sativa

It is well known that the cannabis plant shows a number of chemical varieties: two typical ones are CBDA-rich (CBDA-type) and THCA-rich (THCA-type) varieties[3)]. CBDA-type, which predominantly contains CBDA with no or negligible THCA, is an economically important crop for fiber and seeds. A variety legally cultivated in Tochigi prefecture in Japan belongs to this type.

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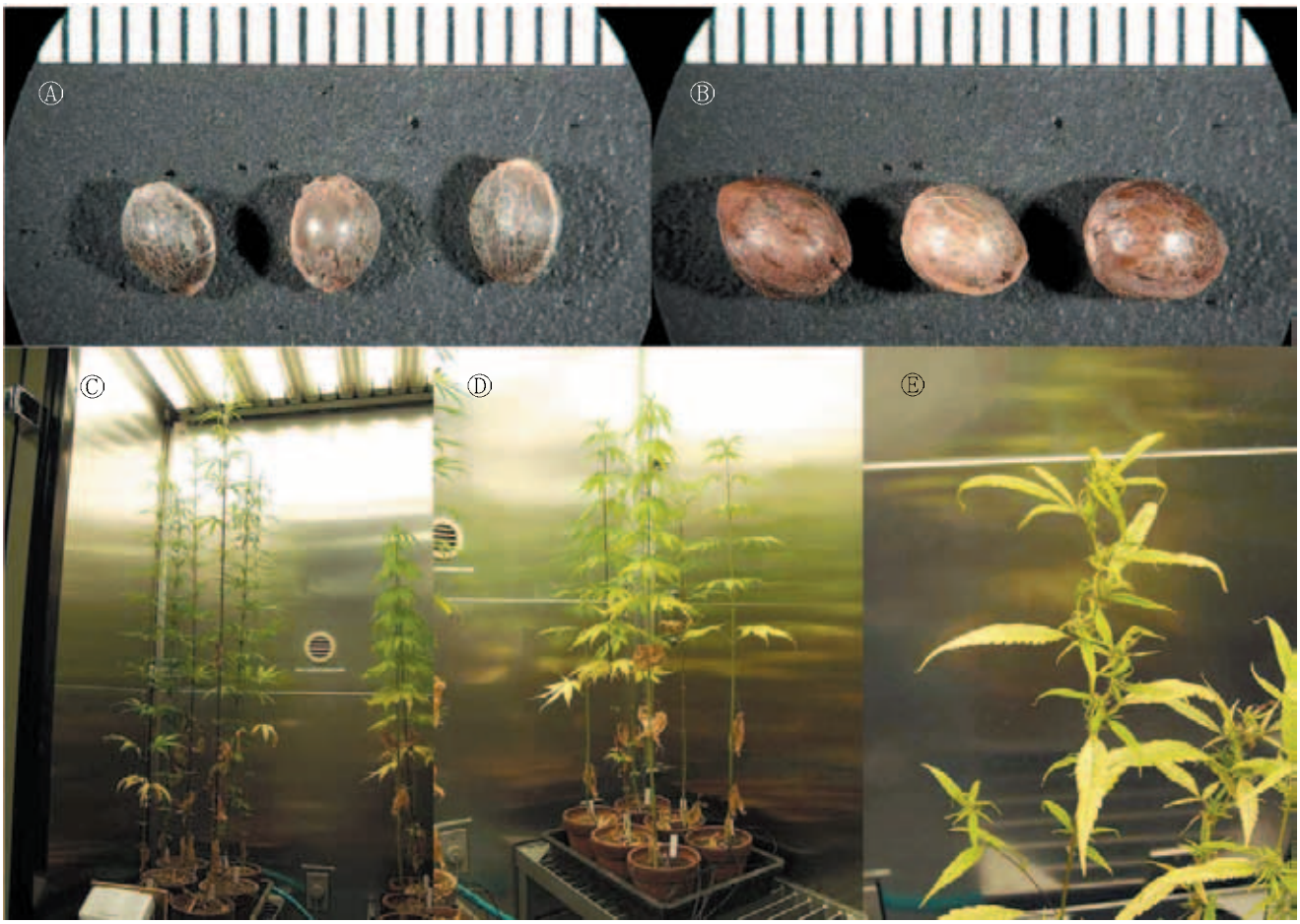

Fig. 2. Photos of cannabis plant
A, seeds of THCA-rich variety; B, seeds of CBDA-rich variety; C, phytotron grown THCA-rich plants; D, phytotron grown CBDA-rich plants; E, a single leaf formed on the THCA-rich plant grown from cutting. A, B: scale shows 1 mm. C, D: 75 days from sowing. E: 94 days from cutting.

THCA-type, which predominantly accumulates THCA, is so-called 'drug type' and cultivated for illegal use. The products of THCA-type are one of the most widely abused illicit drugs in the world and illegal cultivation of the plants poses social problems. In Japan, the plant is illegally cultivated indoor as well as outdoor though the Cannabis Control Act prohibits its cultivation and possession. Criminal investigations of illegal cannabis cultivation require inspection of the growing status. It is significantly important to reveal when the illegal cultivation started and how the plants reached to the present growth status in the investigation. Although many works on identification of cannabis by means of molecular biological techniques[4-10] have so far been published, there were few reports on the growth characteristics of cannabis varieties. In this study, growth characteristics of the two varieties (THCA-type and CBDA-type) are investigated under artificial climate conditions. The growth of THCA-rich variety in the field was also studied.

Materials and Methods

Plant material

The Mexican variety (Exp. code #013, THCA-type) and *cv. CBDA* (Exp. code #045, CBDA-type) of *C. sativa*[7] were used for this study. In a preliminary experiment, they were cultivated in a phytotron and cannabinoids (THCA and CBDA) contents in their leaves were determined as described previously[8]. Both types were used for the growth investigation in a phytotron. For the field study, the THCA-type was used.

Cultivation in a phytotron

The seeds were sown in pots (15 cm i.d., soil-leaf mold-Kureha compost=3:1:1, 6 pots for each variety, 3 seeds/pot) and cultivated in a phytotron (24°C/14 hr light, 20°C/10 hr dark, 60% relative humidity). After germination, the seedlings were thinned out at one plant per pot. Liquid fertilizer (HYPONeX® x 500 solution) was given once a week. Germination, plant height, number of true leaves, number of nodes, number of axillary buds and flow-

ering were periodically observed.

Propagation by cuttings

The axillary shoot cuttings of female plants (*ca*. 10 cm in length) both with or without the main stems were planted in soil (12 cm i.d. pot), and cultivated in a phytotron as mentioned above.

Field cultivation

The seeds of THCA-type were sown 10 cm apart in a bed (1.3 m x 3.7 m), 60 cm ridge width on March 31, 2003. Plant height, number of nodes and flowering were periodically observed. Maximum length and width of the palmate leaf and leaflet, and number of leaflets were determined on Aug. 8, 2003.

Results and Discussion

Comparison of THCA-rich and CBDA-rich varieties

In a preliminary experiment, cannabinoids contents in the leaves were determined. THCA-type contained 3.34-7.51% (average 4.76%) dry weight THCA and 0-0.38% dry weight CBDA, whereas CBDA-type contained 5.08% dry weight CBDA without any detectable THCA. There were differences in color and size of the seeds between the THCA-rich and CBDA-rich varieties (Fig. 2A and B).

The seeds of THCA-type were smaller and darker than those of CBDA-type. There were also differences in germination ability. All the THCA-type seeds germinated in a phytotron while the germination rate of CBDA-type was only 39% (Table 1).

Germination rate of THCA-type was also high in a field (83.%).

Table 1. Germination and male-flowering of cannabis plants cultivated under different environmental conditions

Condition	Variety	Sowing date	Days to germination	Germination rate (%)	Days to male flowering
Phytotron	THCA-type	Jan. 29	6	100	63
	CBDA-type	Jan. 29	6	39	106
Field	THCA-type	Mar. 31	11	83	155

Phytotron conditions: 24°C/14 hr light, 20°C/10 hr dark, and 60% relative humidity. The seeds were sown in 2003.

Both varieties grew healthily in the phytotron (Fig. 2C and D). The period to male-flowering in the phytotron was 63 days for THCA-type and 106 days for CBDA-type (Table 1). However, the period to male-flowering was much longer in the field (THCA-type: 155 days). In the field, the daytime length at the time of sowing (Mar. 31, 2003) was 12 hr 32 min and it peaked (14 hr 39 min) during the summer solstice and then it reduced to 12 hr 57 min at the time of male-flowering (Sep. 2, 2003). On the other hand, the light period was constant at 14 hr in the phytotron; that might be one of the reasons for much shorter period to male-flowering in the phytotron.

Growth characteristics such as plant height, number of true leaves, number of nodes and number of axillary buds were compared between THCA-rich and CBDA-rich varieties (Table 2). All the values of the THCA-type were larger than those of the CBDA-type during the observation period. The THCA-type plants grew to an average height of 125.8 cm and bore fruits after 75 days of cultivation. On the other hand, CBDA-type reached 114.7 cm in height and male-flowered after 106 days of cultivation. Final numbers of true leaves (38.0) and nodes (21.0) of CBDA-type were comparable to those of THCA-type (37.7 and 20.8, respectively). However, there was significant difference in axillary bud formation (24.0 for THCA-type and 17.5 for CBDA-type). These growth and germination differences between the two varieties might be attributed to the genetic variation that has been reported previously[6-8, 10].

Propagation by cuttings

'Drug type' (THCA-rich variety) plants have been experimentally propagated by cuttings[11]. As female plants are of importance for drug production[1], the axillary shoot cuttings of female THCA-type were prepared and planted in soil. Rooting rates of the cuttings either with or without the main stem are shown in Table 3. Plant establishment of the cuttings with the main stem was much easier than those without the main stem. The plant heights are presented in Table 4.

After 94 days, the plants derived from cuttings without the main

Table 2. Growth of cannabis plants in a phytotron

Days	Plant height cm		Number of true leaves		Number of nodes		Number of axillary buds	
	THCA[a]	CBDA[b]	THCA	CBDA	THCA	CBDA	THCA	CBDA
26	25.8 ± 5.1	22.3 ± 7.3	11.0 ± 1.7	9.3 ± 2.1	7.5 ± 0.8	6.7 ± 1.0	6.3 ± 3.2	1.0 ± 1.7
42	65.7 ± 11.6	52.2 ± 9.0	20.3 ± 2.3	15.3 ± 2.1	12.2 ± 1.2	9.7 ± 1.0	17.0 ± 3.3	3.0 ± 3.0
56	93.8 ± 14.9	69.0 ± 8.3	28.3 ± 3.9	19.7 ± 2.3	16.2 ± 1.9	11.8 ± 1.2	22.7 ± 2.4	7.7 ± 6.1
75	125.8 ± 15.7	84.7 ± 8.6	37.7 ± 4.6	23.7 ± 2.9	20.8 ± 2.3	13.8 ± 1.5	24.0 ± 7.3	7.0 ± 6.0
106		114.7 ± 18.3		38.0 ± 7.9		21.0 ± 3.9		17.5 ± 11.5

[a] THCA-type, [b] CBDA-type

The data are shown as mean ± standard deviation (n=6).

Phytotron conditions: 24°C/14 hr light, 20°C/10 hr dark, and 60% relative humidity

Table 3. Rooting (%) of cannabis cuttings

Material	Days of cultivation		
	14	21	64
Axillary shoot without the main stem (n=8)	0	12.5	62.5
Axillary shoot with the main stem (n=4)	50.0	100	100

Cuttings of female plants (*ca*. 10 cm) were planted in soil and cultivated in a phytotron under the same conditions as mentioned above.

Table 4. Plant height (cm) of the female THCA-type plants propagated by cuttings

Material	Days of cultivation	
	64	94
Axillary shoot without the main stem (n=6)	24 ± 23	65 ± 65
Axillary shoot with the main stem (n=4)	33 ± 17	37 ± 16

The data are shown as mean ± standard deviation.

Table 5. Growth of THCA-type cannabis plants in the field

Observation date	May. 5	Jun. 6	Jul. 11	Aug. 7	Sep. 10
Days of cultivation	39	67	102	129	163
Plant height cm	12.8 ± 2.9	80.3 ± 11.3	162.3 ± 18.6	210.7 ± 21.6	260.9 ± 28.2
No. of nodes	4.8 ± 0.6	13.8 ± 0.9	33.4 ± 3.0	48.0 ± 5.4	69.4 ± 9.9

The data are shown as mean ± standard deviation (n=10).
Sowing date: Mar. 31, 2003

Table 6. Comparison of growth rate of THCA-type grown in a phytotron and in the field

Phytotron		Field	
Period (days)	cm/day	Period (days)	cm/day
26-42	2.5	39-67	2.4
42-56	2.0	67-102	2.3
56-75 (flowering)	1.7	102-129	1.8
		129-163 (flowering)	1.5

Table 7. The size of THCA-type cannabis leaves in the field

Parameter	mean ± standard deviation
Maximum palmate leaf length cm	24.2 ± 1.4
Maximum palmate leaf width cm	17.0 ± 1.1
Maximum leaflet length cm	14.1 ± 0.8
Maximum leaflet width cm	2.0 ± 0.3
Number of leaflets	8.3 ± 0.8

The data (n=10) were recorded on Aug. 8, 2003.

stem demonstrated higher average plant height though the standard deviation was larger than the plants derived from the cuttings with the main stem. Female flowers were observed in all the plants grown from cuttings from the early growth stage. In addition, the newly developed leaves were alternate and single instead of palmate (Fig. 2E). The plants grown from the cuttings were in reproduction stage; that might be a reason for the alternated single leaves, because most of nutrition might have been consumed for their reproduction. Pollination of the female flowers on vegetatively-propagated THCA-type plants with the pollens of CBDA-type gave seeds as reported previously[2].

Growth of THCA-type in the field

The THCA-type was cultivated in the field from seeds. Growth parameters of the plants are shown in Table 5. Increase of plant height and number of nodes were most obvious between May and June. Among thirty plants cultivated in the field, 25 plants were female (83.3%). Final plant height on Sep. 10, 2003 was 259.7 ± 20.0 cm for the male plant and 261.4 ± 32.6 cm for the female plant. Despite the great differences in final plant height (average 260.9 cm in the field and 125.8 cm in the phytotron, Tables 2 and 5) and period to male-flowering (155 days in the field and 63 days in the phytotron, Table 1), the growth rates (the increase of plant height per day) were similar in the field and in the phytotron during the vegetative growth stage (2.4 to 1.8 in the field and 2.5 to 2.0 in the phytotron, Table 6).

These results imply that the growth rate (cm/day) at vegetative growth stage is not influenced by the growth environment and might be constant for a variety. Therefore, estimating the starting time of illegal cannabis cultivation might be possible, if the plant is in its vegetative growth stage and the growth rate of the variety has been well studied. Maximum length and width of palmate leaf and number of leaflets were determined on Aug. 8, 2003 and are shown in Table 7. The palmate leaves expanded much larger and more leaflets (average 8.3) were observed in the field when compared to those in the phytotron.

Differences of growth and germination ability between the two varieties found to be obvious in a phytotron. THCA-type, which is used for drug abuse, was more suited for artificial environment

(indoor cultivation) than CBDA-type with showing higher germination rate, faster growth and more vigorous branching. The growth characteristics revealed in this work may offer important information for investigations of illegal cannabis cultivation.

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