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Effects of a Plant Growth Regulator 4,4,4-Trifluoro-3-(indole-3-)-butyric Acid on Thickening Growth of Radish Hypocotyl

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Effects of a synthesized plant growth regulator 4,4,4-trifluoro-3-(indole-3-)-butyric acid (TFIBA) on thickening growth of radish hypocotyls were investigated. The thickening growth of radish hypocotyls, which are the edible part, was stimulated by TFIBA applied to the seeds before transplanting. The fresh weight of the hypocotyls in the seedlings treated with 10^{-4}mol L^{-1} (10^{-4}M) TFIBA solution significantly increased during cultivation in a growth chamber for 28 days as compared to that in the control. However, such significant increase was not observed in the seedlings treated with 10^{-5}mol L^{-1} (10^{-5}M) TFIBA solution. The fresh weight and maximal diameter of hypocotyls in the seedlings treated with TFIBA solution of 10^{-4}M and 10^{-5}M were significantly increased by 30-50% as compared to those of the control at 35 days after transplanting when the seedlings were cultured in a green house under natural light and temperature conditions. The length of hypocotyls in the seedlings treated with 10^{-4}M and 10^{-5}M TFIBA increased approximately 20-50% during the early growth stage as compared to that in the control. The fresh and dry weight of the shoots in the seedlings was apparently increased by treatment with 10^{-4}M and 10^{-5}M TFIBA solutions at 35 days after transplanting. These results suggest that TFIBA stimulates hypocotyl elongation and total photosynthetic activity in the shoots and results in promotion of thickening growth of the hypocotyls.

Key words : hypocotyls, plant growth regulator, radish, thickening growth, trifluoro-indole-butyric acid (TFIBA)

Introduction

The population in the world is increasing rapidly year by year and food production is a very serious problem when considering how to support the increasing population. It is important to develop a cultivation system of plants as well as plant breeding to increase food production effectively. One method for achievement of this purpose is to use plant growth regulators (PGRs) more efficiently to promote plant growth.

4,4,4,-Trifluoro-3-(indole-3-)-butyric acid (TFIBA) (Fig. 1) is a plant growth regulator, which has been newly synthesized[6,7]. TFIBA is an indole derivative with a 4,4,4,-Trifluoro-butyric acid side chain and exhibits many physiological activities on plant growth. For example, TFIBA promotes root elongation of Chinese cabbage and rice, but inhibits *Avena* coleoptile elongation[4,6]. On the other hand, TFIBA stimulates the growth of potato tubers and the thickening growth of radish hypocotyls[4,7]. In this paper, we examined the effect of TFIBA on the growth of radish, especially on the thickening growth of hypocotyls, which are the edible part.

Materials and Methods

Preparation of TFIBA solution

TFIBA was dissolved in 99.5% ethanol at a concentration of 10^{-2}mol L^{-1} and stored at -20°C. Before using, the ethanol was removed thoroughly under reduced pressure performed by sucking with an aspirator (A-3S, Iuchi Co., Japan) and then distilled water was added and it was stirred thoroughly with a sonicator (UT105S, Sharp Co., Japan) for 40 min until the crystals

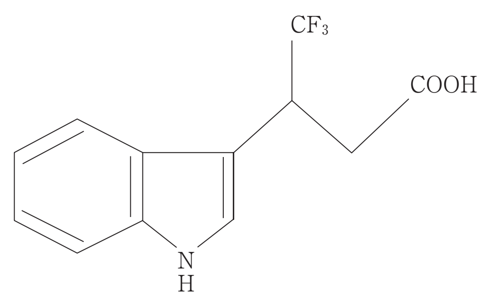

Fig. 1 Chemical structure of 4,4,4-trifluoro-3-(indole-3-) butyric acid (TFIBA).

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of TFIBA were dissolved. Usually water solution of 10^{-3}mol L^{-1} TFIBA was prepared as a solution for use. The sonicated TFIBA solution was diluted with distilled water and was prepared to the solutions of desired concentrations.

Plant Material and culture

Radish (*Raphanus sativus* L. var. *radicula*, cv Akamaru) seeds were germinated on a filter paper in a Petri dish (9 cm in diameter). The Petri dish was supplied 10 mL of TFIBA solution at a concentration of 10^{-5}mol L^{-1} (10^{-5}M) or 10^{-4}mol L^{-1} (10^{-4}M) and incubated at 28 ℃ in the dark for 24 h.

Experiment 1: Cultivation in a growth chamber

The germinated seeds were transplanted to plastic pots (7.5 cm in diameter, 8.5 cm in depth) filled with vermiculite. The plants were cultured in a growth chamber under a day length of 14 h, light quantum of 100 μmol m^{-2} s^{-1}, and a day night temperature regime of 23 ℃/18 ℃. The plants were supplied with distilled water before emergence of foliage leaves and thereafter were fertilized every 3 days with Arnon Hoagland solution containing 8 mmol $NaNO_3$ L^{-1}, 2 mmol P L^{-1} (1.5 mmol KH_2PO_4, 0.5 mmol K_2HPO_4), 10 mmol KCl L^{-1}, 3 mmol $CaCl_2 \cdot 2H_2O$ L^{-1}, 2 mmol $MgSO_4 \cdot 7H_2O$ L^{-1}, 0.05 mmol $FeC_6H_5O_7 \cdot XH_2O$ L^{-1}, 0.01 mmol $MnCl_2 \cdot 4H_2O$ L^{-1}, 0.3 μmol $CuSO_4 \cdot 5H_2O$ L^{-1}, 0.1 μmol $ZnSO_4 \cdot 7H_2O$ L^{-1}, 0.05 mmol H_3BO_3 L^{-1}, and 0.1 μmol $NaMoO_4 \cdot 2H_2O$ L^{-1}. Plants were harvested at one-week intervals after initiation of the thickening growth of hypocotyls and the fresh weight of hypocotyls etc. was measured.

Experiment 2: Cultivation in a green house

Radish plants were grown in a greenhouse at the Faculty of Agriculture, Okayama University under natural light and temperature conditions several times from November 2005 to April 2007. One germinated seed was selected and transplanted into a plastic pot (7.5 cm in diameter, 7 cm in depth) containing vermiculite. Plants were supplied with only tap water alone until foliage leaves were emerged and then supplied with Arnon Hoagland solution for every 3 days. Plants were harvested periodically after initiation of the thickening growth of hypocotyls and the fresh weight, length and diameter of hypocotyls etc. were measured. Then they were dried in a ventilated oven at 65 ℃ for 5 days and the dry weight was also measured.

Results

Effect of TFIBA on the growth of radish seedlings in a growth chamber

Photographs of 5 well-grown whole plants selected from 12 plants and the thickening hypocotyls of 12 plants are shown in Fig. 2. The thickening growth of hypocotyls seemed to be promoted in the plants treated with TFIBA; especially the effect of 10^{-4}M TFIBA seemed to be greater than that of 10^{-5}M TFIBA. The changes in fresh weight of hypocotyls are shown in Fig. 3. The fresh weight of hypocotyls increased to 140% and 132% of the control by treatment with 10^{-4}M TFIBA at 28 and 35 days after transplanting (DAT), respectively. The value in 10^{-4}M TFIBA-treated plants

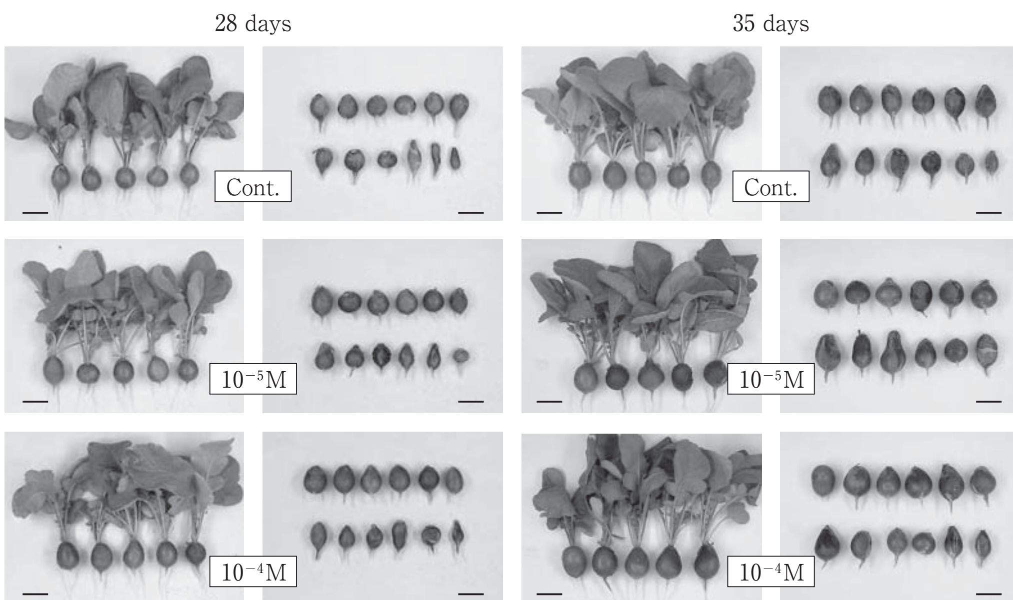

Fig. 2 Photographs of thickening hypocotyls of radish grown in a growth chamber.
Five whole plants with well-grown hypocotyls were selected for photographs (left panels in harvest time). All hypocotyls of 12 plants were subjected to photographs. Bar = 2 cm.

at 28 DAT was statistically significant ($p < 0.05$), but statistical significance was not observed in 35-day-cultivated plants. Slight promotion of thickening growth of hypocotyls was observed at 10^{-5}M TFIBA-treated plants, but statistical significances were not observed in neither 28-day- nor 35-day-cultivated plants. The fresh weights of the shoots and roots were not significantly affected by TFIBA treatment (data not shown).

Effect of TFIBA on the growth of radish seedlings in a green house

Cultivation experiments of radish treated with TFIBA were achieved several times in a green house from November 2005 to April 2007. In this paper, the most recent results are shown. The highest and the lowest temperatures in the green house were measured everyday (Fig. 4). Although the lowest temperature was recorded at below 5°C in the early stage of cultivation, no plants were damaged. The lowest temperature rose to near 10°C in the middle and later stages of cultivation, and plants grew actively. Photographs of thickening hypocotyls of 18 plants harvested at 28, 35 and 42 DAT are shown in Fig. 5. Hypocotyls in the plants treated with 10^{-5}M and 10^{-4}M TFIBA had apparently

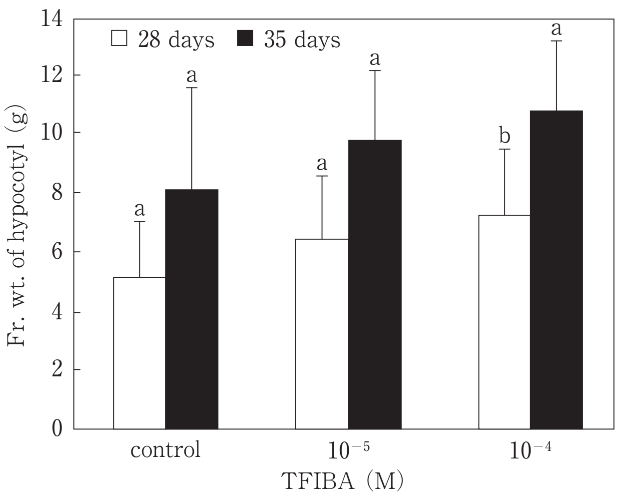

Fig. 3 Effect of TFIBA on thickening growth of radish hypocotyls grown in a growth chamber.
TFIBA was supplied to the seeds during germination for 24 h. Different letters above columns indicate significance at $P < 0.05$. Tukey's test was separately done at 28 days and 35 days. The experiment was repeated three times.

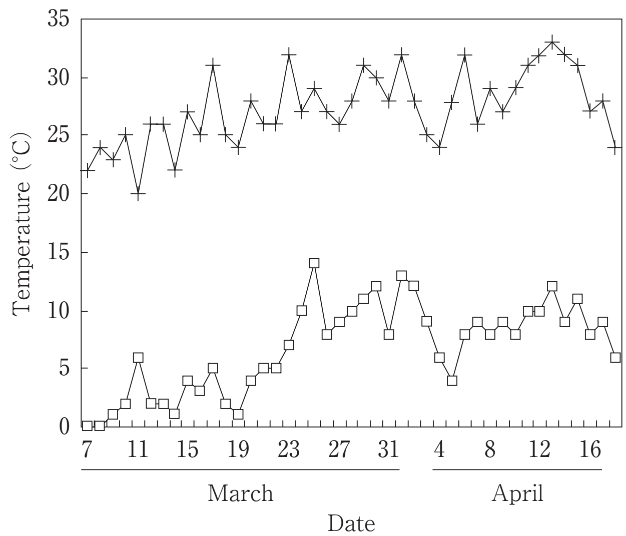

Fig. 4 Changes of temperature in a green house during cultivation of radish plants from March to April 2007.
+: Highest temperature, □: Lowest temperature.

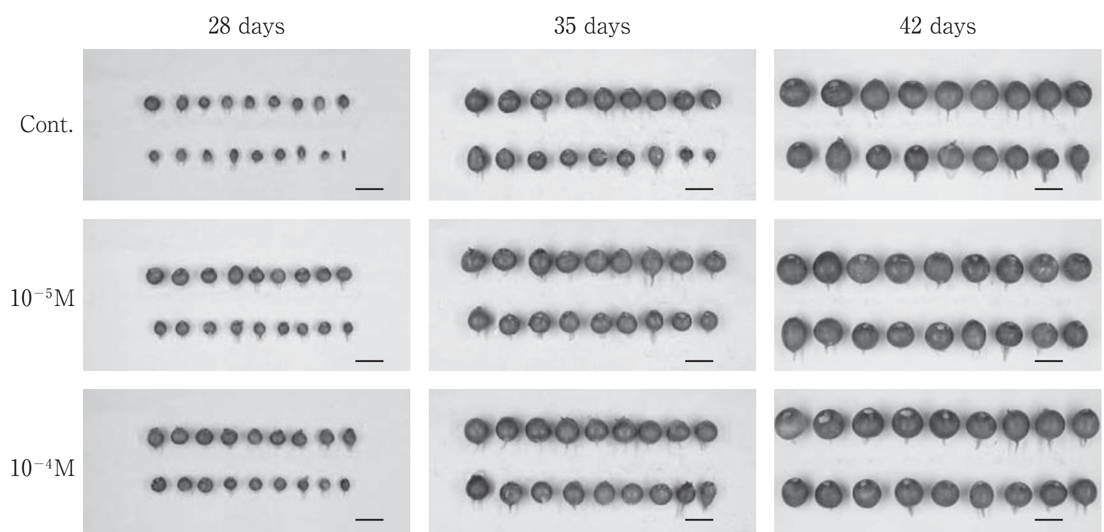

Fig. 5 Photographs of thickening hypocotyls of radish grown in a green house.
The plants were grown in a green house at the Faculty of Agriculture, Okayama University under natural light and temperature conditions. Bar = 2 cm.

thickened more than those in the control plants at 28 DAT. Such a trend was also clearly observed at 35 and 42 DAT. Stimulation of thickening growth of radish seemed to be greater at 10^{-4}M TFIBA treatment than at 10^{-5}M TFIBA treatment (Fig. 5).

The fresh and dry weights of hypocotyls, diameter of hypocotyls, shoot and root weights are shown in Figs. 6, 7 and 8. The fresh weight of hypocotyls increased to 239%, 153% and 124% of the control in the plants treated with 10^{-4}M TFIBA, and 206%, 132% and 117% in the plants treated with 10^{-5}M TFIBA at 28, 35 and 42 DAT, respectively (Fig. 6). The diameters of thickening hypocotyls significantly increased 20–30% in both plants treated with 10^{-4}M and 10^{-5}M TFIBA until harvesting time of 35 DAT (Fig. 7). Although statistical significance was not observed in the plants treated with 10^{-5}M TFIBA at 42 DAT, it was still observed in the plants treated with 10^{-4}M TFIBA (Fig. 7). The fresh weights of the shoots in the plants treated with 10^{-4} and 10^{-5}M TFIBA increased 20–30% significantly compared to that of the control (Fig. 8). Leaf number in the plants treated with 10^{-4}M TFIBA also significantly increased (Table 1). However, the fresh weight of the roots was not increased by TFIBA treatment (Fig. 8).

Discussion

One of the most drastic effects of TFIBA is stimulation on rice root elongation. When rice seedlings were grown in a 10^{-5}M TFIBA solution the roots were elongated to near 200% of the untreated control[6]. Moreover, it is indicated that TFIBA is an effective plant growth regulator to increase plant production as stimulation of the growth of potato tubers or the thickening growth of radish hypocotyls[4,7]. In this study, we focused on the effect of TFIBA on the thickening growth of radish hypocotyls. When the seeds were germinated in a Petri dish containing 10^{-4} or 10^{-5}M TFIBA solution for 24 h, then transplanted to the pots containing vermiculite and cultivated, the thickening growth of hypocotyls was apparently promoted (Figs. 2, 3, 5 and 6). The increase of the fresh weights of hypocotyls by TFIBA treatment occurred more remarkably in seedlings cultivated in a green house than those in a growth chamber. The main cause can be attributed to supply of photosynthetic

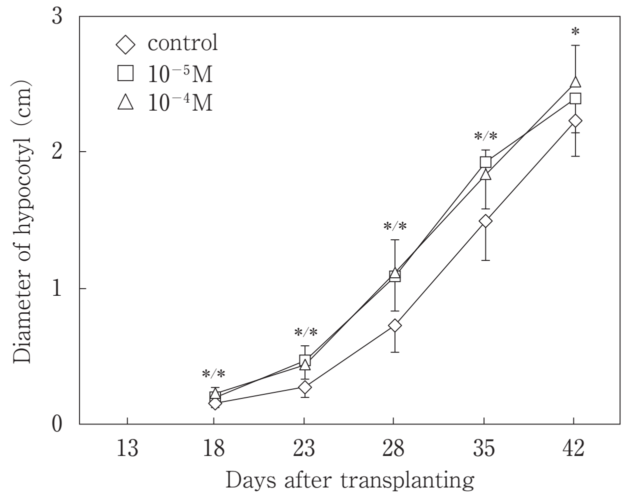

Fig. 7 Effects of TFIBA on diameter of radish hypocotyls.
Statistical analysis (Tukey's test) was done separately at each harvest time. The marks of */* and * indicate significance at $P<0.05$ in both of 10^{-4} and 10^{-5}M TFIBA treatment and only in 10^{-4}M TFIBA treatment, respectively. Vertical bars indicate ± SD (n = 18).

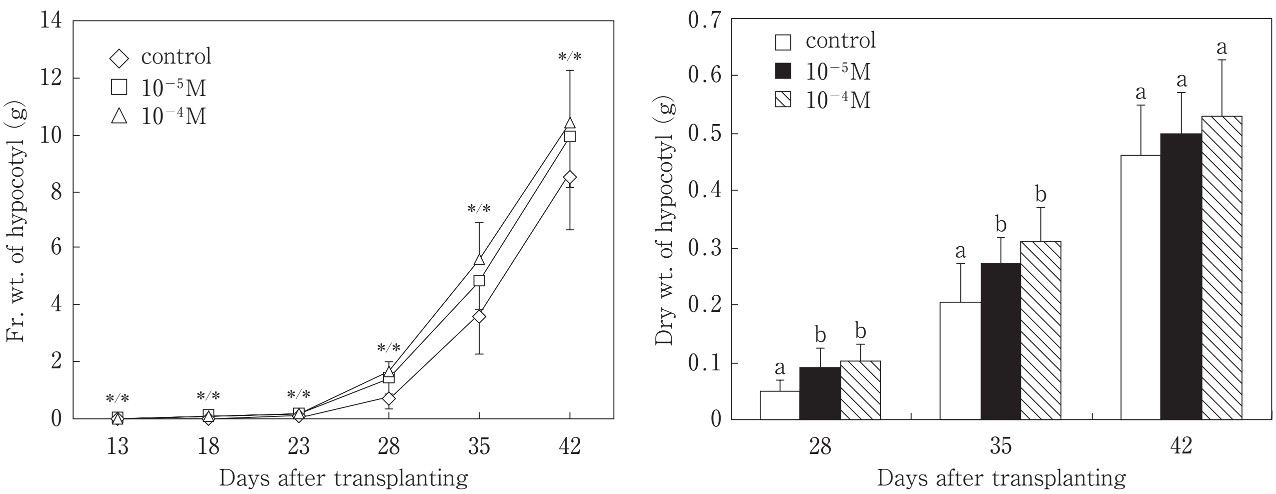

Fig. 6 Effects of TFIBA on fresh and dry weights of radish hypocotyls.
Statistical analysis (Tukey's test) was done separately at each harvest time. The mark of */* indicates significance at $P<0.05$ in both of 10^{-4} and 10^{-5}M TFIBA treatment compared to the control. Different letters above columns indicate significance at $P<0.05$. Vertical bars indicate ± SD (n = 18).

products. Light quantum in the growth chamber was too low (see Materials and Methods) to support the thickening growth of hypocotyls elongated by TFIBA. Actually, the hypocotyls of many of the plants were not a good spherical shape and had a vertically elongated irregular shape (Fig. 2). In contrast, light quantum, which was not measured, in a green house should be enough to support the thickening growth of hypocotyls elongated by TFIBA, and almost all of the thickened hypocotyls had a good spherical shape (Fig. 5). The fresh weight of shoots and leaf number in the seedlings treated with 10^{-4} or 10^{-5}M TFIBA could be seen have increased in a green house (Fig. 8 and Table 1). These results indicate that the effect of TFIBA is primarily to elongate radish hypocotyls and subsequently to establish photosynthetic systems to support the thickening growth of hypocotyls. Hayata et al.[1-3] investigated the relationships between development of radish hypocotyls and light intensity, temperature or internal hormonal levels etc.. The thickening growth of radish hypocotyls was markedly inhibited under low light intensity, but the diameter of hypocotyls was significantly increased and the elongation of hypocotyls was inhibited by application of kinetin[1]. Further, cytokinin applied directly to the

Table 1 Effect of TFIBA on leaf number of radish grown in a green house

TFIBA treatment	Leaf number 18 (Days after transplanting)	23	28	35	42
Untreated	2.0a	3.7a	4.8a	5.6a	6.5a
10^{-5}M	2.6b	3.9a	5.3ab	5.9ab	6.9a
10^{-4}M	2.9c	4.1a	5.4b	6.3b	7.6b

Statistical analysis was done at respective harvest times with Tukey's test.
Different letters indicate significance at $p<0.05$ (n = 18).

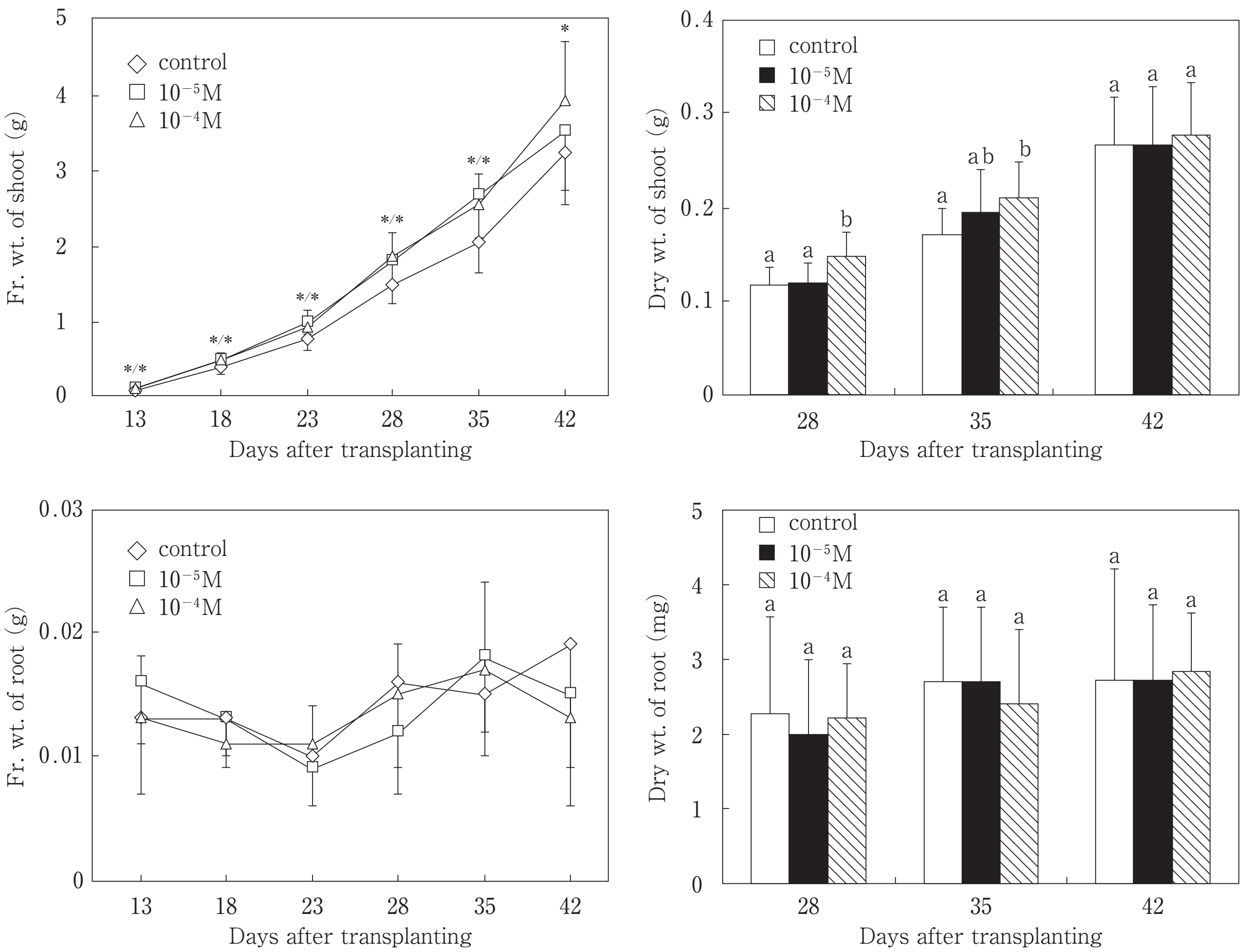

Fig. 8 Effects of TFIBA on growth of radish shoots and roots.
Statistical analysis (Tukey's test) was done separately at each harvest time. The marks of */* and * indicate significance at $P<0.05$ in both of 10^{-4} and 10^{-5}M TFIBA treatment and only in 10^{-4}M TFIBA treatment, respectively. Different letters above columns indicate significance at $P<0.05$. Vertical bars indicate ± SD (n = 18).

hypocotyls by injection promoted the thickening growth of the hypocotyls and the elongation was inhibited under high temperature conditions[2]. These results suggest that hormone(s) including cytokinin relate to the thickening growth of radish hypocotyls. As our data indicated, TFIBA is also a potent plant growth regulator to promote the thickening growth of radish hypocotyls. However, the action mode of TFIBA appeared to be primarily different from that of cytokinin. TFIBA promoted radish hypocotyl elongation even under high light intensity in a green house and also increased the diameter of hypocotyls (Fig. 7). These facts indicate that TFIBA is a very attractive and useful plant growth regulator to increase the productivity of root vegetables, as pointed out by Katayama[4] and Kato et al.[7].

The preparation of TFIBA solution is somewhat troublesome. TFIBA is practically insoluble in water. Therefore, to prepare the applicable TFIBA solution to plants, TFIBA is dissolved in ethanol, followed by vacuum drying to remove the ethanol thoroughly, and then dissolved in water thoroughly by stirring vigorously with a sonicator (see Materials and Methods). Zhang and Hasenstein[8] reported that 100 μM TFIBA dissolved in an organic solvent DMSO (dimethyl sulfoxide) promoted elongation of primary roots by 40% in 72 h but inhibited hypocotyls growth by 35% in lettuce (*Luctuca sativa* L.) . They also described that root growth was not affected by DMSO which concentration is less than 0.5% (v/v)[8]. We examined the effectiveness of DMSO as a solvent of TFIBA. DMSO below 0.5% (v/v) did not affect growth of rice and maintained root elongation effect of TFIBA in rice (data not shown). Promotion of thickening growth of radish hypocotyls occurred in the seedlings treated with 10^{-4} or 10^{-5}M TFIBA dissolved in 0.25% DMSO as well as TFIBA dissolved in water (data not shown). However, expression of effect of TFIBA was more stable, reproducible, and somewhat greater in TFIBA dissolved in water. For these reasons, we mainly used TFIBA dissolved in water.

In this experiment, the quality of the thickening hypocotyls stimulated by TFIBA was not examined. The content of sugar, minerals and vitamins etc. is important to estimate the quality of vegetables. The content of these compounds must be determined to confirm the effectiveness of TFIBA for plant production. We plan to further clarify TFIBA effect on thickening growth of radish from the aspects of morphology and molecular biology. Further, we will examine the effect of TFIBA on the growth in many plants and clarify a whole range of effects of TFIBA on plant growth.

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ハツカダイコンの胚軸肥大成長に対する
トリフルオロインドール酪酸の効果

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ハツカダイコンの肥大成長に対する合成植物成長調節物質トリフロオロインドール酪酸（TFIBA）の効果について調べた．種子を10^{-4}ないしは10^{-5}M TFIBA 溶液に24時間浸漬し，発芽種子をバーミキュライトの入ったポットに移植して，14時間日長，昼夜温度23℃/18℃のグロスチャンバーで栽培すると，明らかな胚軸の肥大成長促進が認められた．肥大成長は10^{-4}M TFIBA 処理の植物体でより顕著に現れた．同様に処理した発芽種子を自然光，無加温のガラス室で，2007年３月から４月にかけて栽培すると，胚軸の肥大成長はグロスチャンバーでの栽培に比べて，より顕著に現れた．35日目の肥大胚軸部の新鮮重は，10^{-4}Mと10^{-5}M のいずれの濃度においても30-50％増加した．TFIBA 処理によって生育初期段階で胚軸が20-50％長くなり，また葉数が増えて地上部重が有意に重くなり，胚軸の肥大部径も有意に大きくなった．これらの結果は，TFIBA が胚軸の初期成長を促進し，生育の進行と共に光合成器官である葉組織を増加させ，胚軸の肥大成長を促進したことを示している．可食部である胚軸肥大部の形状は，ガラス室ではきれいな球状となるものが多いのに比べて，グロスチャンバーでは縦長のいびつな形をしたものが多くなった．これは，グロスチャンバーでは光量が不足し，十分な光合成産物が胚軸の肥大に供給されなかったためと考えられた．

本研究で，TFIBA を発芽時に浸漬投与するだけでハツカダイコンの可食部（胚軸）の肥大成長を有意に促進させることが明らかとなった．得られた結果は，TFIBA がハツカダイコンをはじめとする根茎野菜の生産促進に有効な植物成長調節物質であることを示している．