低温および長日によるダイコンの抽台・花成における内生ジベレリンの役割

誌名	園藝學會雑誌
ISSN	00137626
著者	西島, 隆明 桂, 直樹 腰岡, 政二
巻/号	67巻3号
掲載ページ	p. 319-324
発行年月	1998年5月

園学雑. (J. Japan. Soc. Hort. Sci.) 67(3) : 319-324. 1998.

Role of Endogenous Gibberellins in Cold-Induced Stem Elongation and Flowering of Japanese Radish (*Raphanus sativus* L.)

Takaaki Nishijima[1], Naoki Katsura[1]*, Masaji Koshioka[1], Hiroko Yamazaki[1], Masayoshi Nakayama[2]**, Hisakazu Yamane[2], Isomaro Yamaguchi[2], Takao Yokota[2]***, Noboru Murofushi[2], Nobutaka Takahashi[2]****, Mizuo Nonaka[3] and Lewis N. Mander[4]

[1]*National Research Institute of Vegetables, Ornamental Plants and Tea, Ano-cho, Mie 514-2328*

[2]*Faculty of Agriculture, The University of Tokyo, Bunkyo-ku, Tokyo 113-0032*

[3]*Kurume Branch, National Research Institute of Vegetables, Ornamental Plants and Tea, Kurume-shi, Fukuoka 839-0851*

[4]*Research School of Chemistry, The Australian National University, G. P. O. Box 4, Canberra A. C. T. 2601, Australia*

Summary

Eighteen endogenous gibberellins (GAs) which belong to the early 13-hydroxylation pathway and the early non-hydroxylation pathway of GA-biosynthesis were identified by combined gas chromatography/mass spectrometry (GC/MS) in shoots of a cold-requiring plant, *Raphanus sativus* L. Contents of GA_1 and GA_4, the probable endogenous biologically-active GAs, increased in the stem during a long-day condition (LD) both with or without a prior cold treatment (CT); however, the increase was greater with CT than without it. In contrast to the contents of 3β-hydroxylated GAs (i.e. GA_1 and GA_4), that of GA_{34}, a 2β-hydroxylated GA, was decreased significantly by CT and LD, indicating that CT and LD activated the 3β-hydroxylation and inactivated the 2β-hydroxylation during the course of GA-biosynthesis. GA_1 and GA_4 contents in the leaf decreased during LD subsequent to CT, whereas they increased with LD alone. The increase in GA_1 and GA_4 contents in the stem probably promoted the cold-induced stem elongation and flowering of *R. sativus*.

Key Words: flowering, gibberellin, *Raphanus sativus* L., stem elongation, vernalization.

Introduction

Japanese radish (*Raphanus sativus* L.) is a cold requiring plant (CRP) whose cultivars differ greatly in cold requirement for stem elongation (bolting) and flowering (Kagawa and Sata, 1957). Undesirable bolting of the plant by unusual climatic changes sometimes reduces taproot enlargement.

It is generally concluded that endogenous gibberellins (GAs) play a regulatory role in cold-induced stem elongation of CRPs (Zeevaart, 1983; Pharis and King, 1985). However, participation of GAs in cold-induced flowering of CRPs is still in dispute, because application of exogenous GA and GA-biosynthesis inhibitor did not affect flowering of many CRPs (Zeevaart, 1983; Pharis and King, 1985).

In *R. sativus*, application of a triazol GA-biosynthesis inhibitor greatly retarded not only cold-induced stem elongation but also flowering, and the retardations were reversed by exogenous application of GA_3 (Nishijima et al., 1997). These results indicate that GA is involved not only in cold-induced stem elongation but also in the flowering of this plant.

Furthermore, Nakayama et al. (1995) using radioimmunoassay found that GA_1 and GA_4 contents in the stem were higher at anthesis than at the vegetative stage.

In this study, we investigated the changes in endogenous GA contents during cold-induced stem elongation and flowering of *R. sativus* using combined gas chromatography/mass spectrometry (GC/MS). The role of endogenous GAs in cold-induced stem elongation and flowering is discussed.

Received; February 7, 1997. Accepted; July 16, 1997.
This work was supported by a research grant (Biomedia Projects BMP-97-V-1-4) from the Ministry of Agriculture, Forestry and Fisheries (Japan).

*Present address : National Institute of Biological Resources, Tsukuba, Ibaraki 305-8602, Japan.

**Present address : National Research Institute of Vegetables, Ornamental Plants and Tea, Ano-cho, Mie 514-2328, Japan.

***Present address : Department of Biosciences, Teikyo University, Utsunomiya, Tochigi 320-0032, Japan.

****Present address : The Insitute of Physical and Chemical Research (RIKEN), Wako-shi, Saitama 351-0100, Japan.

Materials and Methods

Plant Material

Seeds of *Raphanus sativus* L. (cv. Taibyo-sobutori, Takii Seed Co.) were sown in plastic containers (50×35 cm, 7 cm in depth) filled with a horticultural medium (Kureha-engei-baido, Kureha Chemical Co.). As shown in Fig. 1, the plants were grown under four combinations of temperature and photoperiodic conditions (i.e., S, L, C/S and C/L). The plants grown under the respective conditions were called S plant and so on. The temperature was kept at 20°C throughout the growth period except during the cold treatment (CT). The C/S and C/L plants were both grown under a short day condition (SD) for 15 days from sowing. SD consisted of 8 h irradiation from metal-halide lamps (470 μmol · m^{-2} · s^{-1}), followed by 16 h dark period. Then the plants were subjected to 20 days of CT at 5°C. During CT, the lighting condition consisted of 8 h irradiation from fluorescent lamps (14 μmol · m^{-2} · s^{-1}), followed by 16 h dark period. After CT, the plants were grown under SD for 6 days to avoid the change in endogenous GA content caused by severe temperature shift (Nishijima et al. unpublished data). In contrast, the S or L plants were grown for 21 days under SD from planting. Those cold-treated (C/S and C/L) and nontreated (S and L) plants were then subjected to 15 days of photoperiodic treatment. During the photoperiodic treatments, the plants were exposed to the above SD or a long-day condition (LD). LD consisted of the same main light period as that of SD, but supplemented with subsequent 8 h weak light from incandescent lamps (10 μmol · m^{-2} · s^{-1}) to minimize the difference in total radiation between SD and LD. Days were counted from the beginning of the photoperiodic treatment as shown in Fig. 1. S, L, C/S and C/L plants had approximately the same number of expanded leaves at the beginning of the photoperiodic treatments.

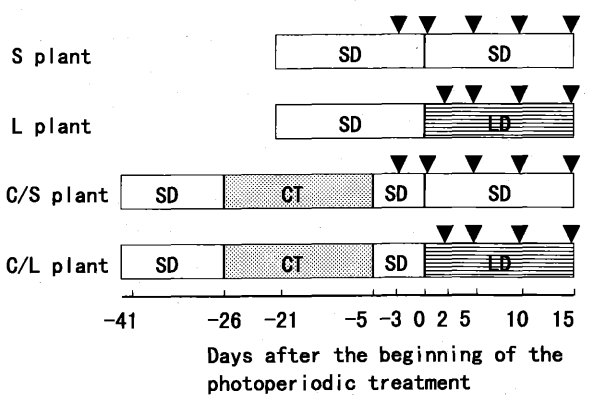

Fig. 1. Scheme of the cold and photoperiodic treatments for preparation of sample plants for gibberellin analysis. CT : cold treatment (5°C; 8 h day at 14 μmol · m^{-2} · s^{-1} by fluorescent lamps), SD : short day condition (8 h day at 470 μmol · m^{-2} · s^{-1} by metal-halide lamps; 20 °C), LD : long day condition (16 h day consisted of the same 8 h main light period as that of SD and the following 8 h weak light period at 10 μmol · m^{-2} · s^{-1} by incandescent lamps; 20 °C). ▼ : sample collection.

Leaves and the stem including ca. 1 cm of hypocotyl were collected for GA analysis at the times indicated by triangles in Fig. 1. The samples were immersed in 80% aqueous methanol (MeOH) and then stored at −20 °C until they were extracted. On sampling dates, the stem lengths of the plants were measured, and the floral stage was identified by observing the apical meristem under a dissecting microscope and scored based on the criteria by Eguchi and Koide (1944).

Analysis of endogenous GAs

Methods for identification and quantification of endogenous GAs using GC/MS and ^{2}H-labeled GAs as internal standards were described previously (Nishijima et al., 1995). Quantities of endogenous GAs were calculated from the ion peak area ratios of endogenous GAs against ^{2}H-labeled GAs. A sample of 150–200 gFW and 50–60 gFW were used for identification and quantification of GAs, respectively. GA-analysis was conducted singly.

Results

Effects of CT and day length on stem elongation and flowering

Shoots of unchilled S and L plants and the C/S plants did not elongate nor flower (Fig. 2). However, stems of C/L plants elongated and formed flower buds from ca. 10 days after beginning of LD, achieving full anthesis 22–29 days after the onset of LD treatment.

Identification of endogenous GAs

Eighteen endogenous GAs, namely, GA_1, GA_4, GA_9, GA_{15}, GA_{17}, GA_{19}, GA_{20}, GA_{24}, GA_{34}, GA_{40}, GA_{44}, GA_{47}, GA_{51}, GA_{81}, 3-*epi*-GA_1, 3-*epi*-GA_4, GA_{20} 15-ene and GA_{24} 15-ene were identified. Out of these GAs, five were not identified in the previous experiment (Table 1 and Nakayama et al., 1995).

Effects of CT and day length on endogenous GA contents

Figure 3 shows the effects of CT and day length on GA_1 contents in stem and leaf of *R. sativus*. In S and L plants, stem GA_1 content was higher under LD than under SD, whereas in C/L plants where CT was applied before LD, the GA_1 content in the stem was even higher, beginning to increase 2 days after the onset of the LD treatment. This increase preceded stem elongation and flower bud formation (Fig. 2). Contrarily, GA_1 content in stems of C/S plants did not substantially change until the end of the photoperiodic treatment.

LD kept leaf GA_1 content higher than SD in the unchilled plants (Fig. 3, S and L plants), whereas in the

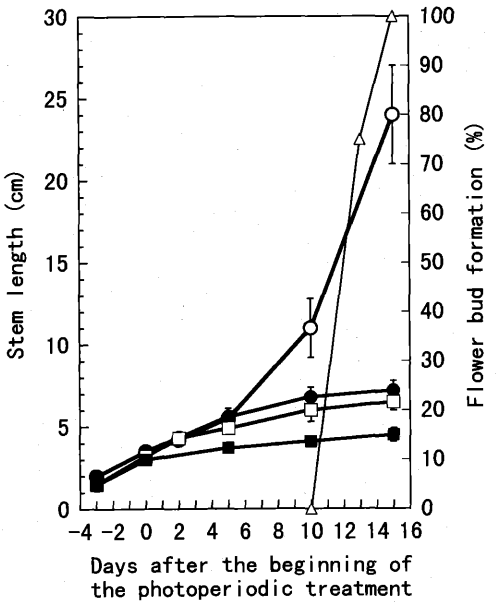

Fig. 2. Curves illustrating the effects of cold and photoperiodic treatments on stem elongation and flowering of *Raphanus sativus* cv. Taibyo-sobutori. The treatments conducted on the S (■), L (□), C/S (●) and C/L (○) plants are shown in Fig. 1. △ : flower bud formation in C/L plants. Vertical bars represent±SE (n=10).

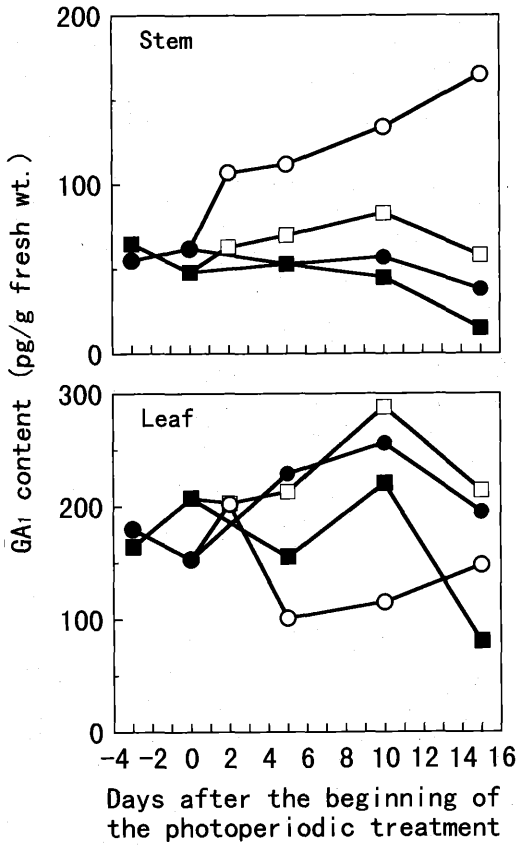

Fig. 3. Changes in endogenous GA_1 content in *Raphanus sativus* cv. Taibyo-sobutori subjected to cold and photoperiodic treatments. The treatments conducted on the S (■), L (□), C/S (●) and C/L (○) plants are shown in Fig. 1.

Table 1. Gibberellins which were newly identified in the present experiment from shoot of *Raphanus sativus*.

Gibberellin[z]	HPLC[y]-fraction[x]	KRI[w]	Principal ions and relative intensity (% of base peak)
GA_{40}[v]	17	2503	418(0), 403(8), 387(6), 371(100), 343(75)
GA_{47}[v]	19-20	2618	506(100), 491(1), 474(3), 459(12), 431(5)
GA_{81}	19-20	2653	506(100), 491(3), 477(5), 448(1), 416(7)
GA_{20} 15-ene[v]	17	2422	418(100), 403(11), 389(63), 359(68), 313(8)
GA_{24} 15-ene[v]	25-26	2390	374(1), 346(6), 342(2), 314(100), 286(76)

[z] Gibberellins listed in this table were not identified in the previous experiment (Nakayama et al., 1995)
[y] High-performance liquid chromatography.
[x] Sample was purified with a reverse-phase C_{18}-HPLC as described previously (Nishijima et al., 1995), and the HPLC-fractions which showed significant biological activity to the sensitized rice seedling bioassays (Nishijima and Katsura, 1989; Nishijima et al., 1993) were subjected to GC/MS analysis.
[w] Kovats' Retention Index.
[v] Tentative identification [i.e. identification by comparison of mass spectra and KRI of GAs with the data by Gaskin and MacMillan (1991)]

C/L plants, LD decreased GA_1 content in the leaf 5 days after beginning of the photoperiodic treatment simultaneously with its increase in the stem.

Changes in content of stem GA_4, the probable biologically-active GA of the early non-hydroxylation pathway, were similar to those of GA_1 (Fig. 4), namely, LD increased stem GA_4 content in L plants, and the increase was greater in C/L plants. The increase in stem GA_4 content of C/L plant preceded stem elongation and flowering. Furthermore, LD decreased leaf GA_4 content in the C/L plants, whereas LD increased the GA_4 level in the unchilled L plants.

Figure 5 shows the contents of 9 GAs in S and C/L plants. These GAs are located on the main streams of the early 13-hydroxylation and the early non-hydroxylation pathways. The samples were collected 5 days after the beginning of the photoperiodic treatment, when marked increase in GA_1 and GA_4 contents in the stems occurred in C/L plants (Figs. 3 and 4). CT and LD markedly dcecreased GA_{34} and GA_{15} contents and increased GA_1, GA_4 and GA_{20} contents. Contents of the other GAs were increased slightly by CT and LD.

Discussion

Most of GAs identified from *R. sativus* cv. Taibyo-sobutori were the same as those identified previously (Nakayama et al., 1995), so that both the early 13-hydroxylation and the early nonhydroxylation pathways apparently function in the shoot (Fig. 6). However, GA_{40}, GA_{47}, GA_{81}, GA_{20} 15-ene and GA_{24} 15-ene were newly identified (Table 1), whereas GA_{12}, GA_{25}, GA_{29} and 12 β-hydroxy GA_{24} were not detected in the present study. These differences might have resulted

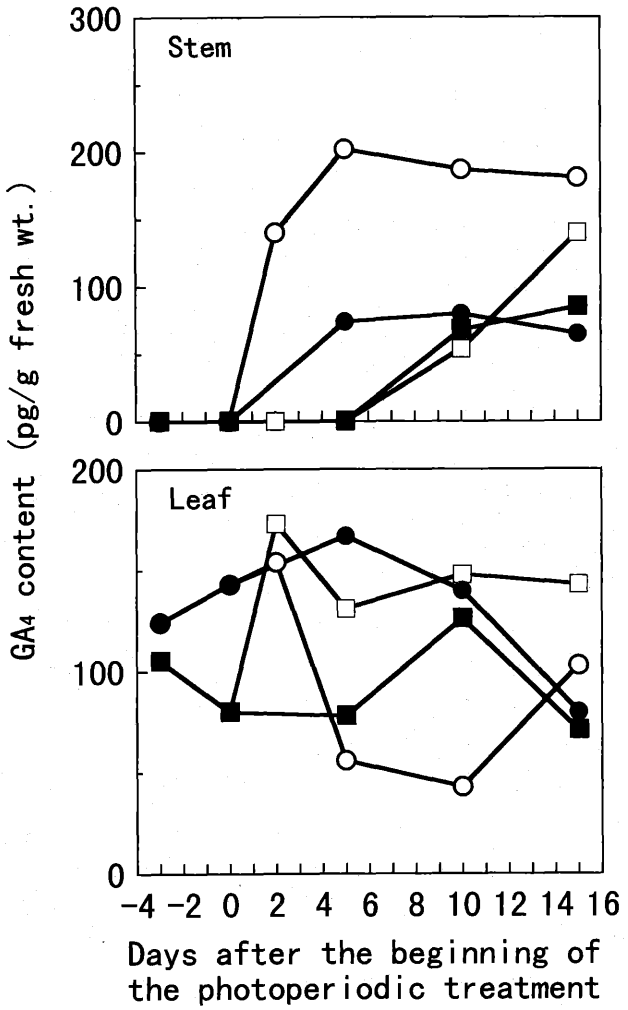

Fig. 4. Changes in endogenous GA_4 content in *Raphanus sativus* cv. Taibyo-sobutori subjected to cold and photoperiodic treatments. The treatments conducted on the S (■), L (□), C/S (●) and C/L (○) plants are shown in Fig. 1.

from the difference in plant age, because younger plants were used in this experiment.

In a few CRPs, analysis of GAs using GC/MS revealed that content or metabolism of endogenous GAs was increased by cold induction (Hasebroek et al., 1993; Zenewich and Rood, 1995). In this experiment

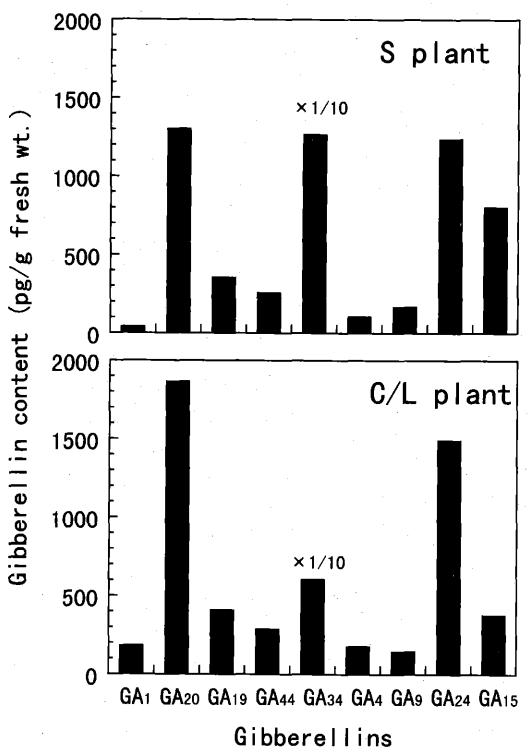

Fig. 5. Endogenous gibberellin contents in S and C/L plants subjected to cold and photoperiodic treatments as shown in Fig. 1. Concentration of GA_{34} is shown as 1/10 magnitude of the actual concentration.

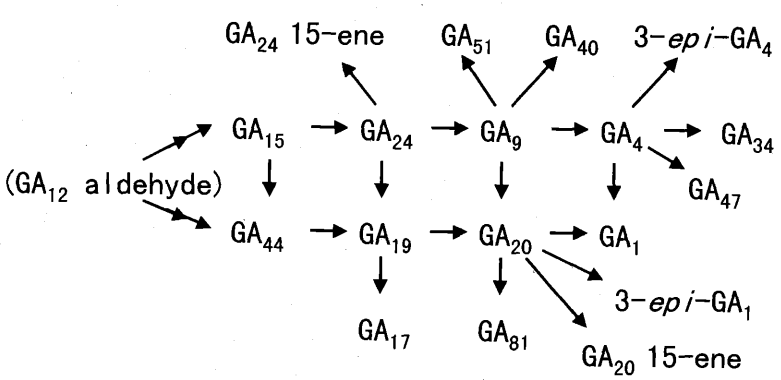

Fig. 6. Hypothetical biosynthetic pathways of the gibberellins identified in shoot of *Raphanus sativus* cv. Taibyo-sobutori. GA_{12} aldehyde was possibly present, but it was not detected.

with *R. sativus,* cold induction and LD increased GA_1 and GA_4 contents in stems (Figs. 3 and 4). The increase under LD was greater in cold-treated plants than in cold-nontreated plants. In the previous experiment (Nishijima et al., 1997), uniconazole, a GA-biosynthesis inhibitor, retarded not only cold-induced stem elongation but also flowering of *R. sativus,* and the inhibition was restored by exogenous GA_3. These results suggested involvement of GAs not only in stem elongation but also in flowering of *R. sativus*. The increase in GA_1 and GA_4 contents in stem by CT and LD might cause not only stem elongation but also flowering of *R. sativus*.

In contrast to stem, leaf GA_1 and GA_4 contents of the cold-treated plants decreased drastically 5 days after transferring to LD (Figs. 3 and 4). Thus stem seems to serve as a "sink" for GAs when cold-induced stem elongation and flowering occur.

In CRPs, *Brassica napus* and *Thraspi arvense,* the GA-biosynthetic steps prior to GA_{44} were activated by CT (Hasebroek et al., 1993; Zenewich and Rood, 1995). In *R. sativus,* GA_1 and GA_4 contents were significantly increased by CT and LD, while their precursors, GA_{20} and GA_9, showed a slight increase and a decrease, respectively (Fig. 5). Thus, 3 β-hydroxylation was probably activated by CT and LD. Furthermore, the large decrease in GA_{34} content may indicate the inactivation of the 2 β-hydroxylation by CT and LD.

CT and LD may also affect 'tissue sensitivity' to GAs. In several CRPs, LD enhanced the exogenous GA-induced stem elongation (Bernier et al., 1981). In *R. sativus,* LD enhanced the exogenous GA_3-induced stem elongation and flowering (Suge and Rappaport, 1968). Furthermore, Nishijima et al. (1997) found that exogenous GA_3 induced stem elongation and flowering of the *R. sativus* plants, whose endogenous GA-biosynthesis was blocked by a GA-biosynthesis inhibitor, were markedly enhanced by CT and LD. Those results indicate that CT and LD confer the increase in tissue sensitivity to GA together with an increase in contents of endogenous biologically-active GAs. Thus, the increase in the 'GA-sensitivity' and 'GA-content' caused by CT and LD might interact to promote stem elongation and flowering of *R. sativus*.

Acknowledgment

The authors thank Mrs. Araki for her assistance.

Literature Cited

Bernier, G., J. M. Kinet and R. M. Sachs. 1981. The physiology of flowering. Vol. II, Transition to reproductive growth. p. 93-133. CRC Press, Boca Raton, Florida.

Eguchi, Y. and M. Koide. 1944. Studies on the relationship among sowing date, flower initiation and vernalization of *Raphanus sativus* L. and *Brassica campestris* L. J. Japan. Soc. Hort. Sci. 15 : 1-27. (in Japanese).

Gaskin, P. and J. MacMillan. 1991. GC-MS of the gibberellins and related compounds : Methodology and a library of spectra. p. 132-530. Cantock's Enterprises, Bristol, UK.

Hasebroek, J. P., J. D. Metzger and E. R. Mansager. 1993. Thermoinductive regulation of gibberellin metabolism in *Thraspi arvense* L. II. Cold induction of enzymes in gibberellin biosynthesis. Plant Physiol. 102 : 547-552.

Kagawa, A. and M. Sata. 1957. Studies on the effect of low temperature induction in radish plant, II. On the stage and varietal differences in Japanese radishes. Res. Bull. Fac. Agr. Gifu Univ. 8 : 57-66. (In Japanese).

Nakayama, M., H. Yamane, H. Nojiri, T. Yokota, I. Yamaguchi, N. Murofushi, N. Takahashi, T. Nishijima, M. Koshioka, N. Katsura and M. Nonaka. 1995. Quantitative and qualitative analysis of endogenous gibberellins in *Raphanus sativus* L. during cold treatment and the subsequent growth. Biosci. Biotech. Biochem. 59 : 1121-1125.

Nishijima, T. and N. Katsura. 1989. A modified micro-drop bioassay using dwarf rice for detection of femtomol quantities of gibberellins. Plant Cell Physiol. 30 : 623-627.

Nishijima, T., M. Koshioka, H. Yamazaki. 1993. A highly-sensitive rice seedling bioassay for the detection of femtomole quantities of 3 β-hydroxylated gibberellins. Plant Growth Regul. 13 : 241-247.

Nishijima, T., M. Koshioka, H. Yamazaki, H. Miura and L. N. Mander. 1995. Endogenous gibberellins and bolting in cultivars of Japanese radish. Acta Hortic. 394 : 199-206.

Nishijima, T., N. Katsura, M. Koshioka, H. Yamazaki and L. N. Mander. 1997. Effects of uniconazole and GA_3 in cold-induced stem elongation and flowering of *Raphanus sativus* L. Plant Growth Regul. 21 : 207-214.

Pharis, R. P. and R. W. King. 1985. Gibberellins and reproductive development in seed plants. Ann. Rev. Plant Physiol. 36 : 517-568.

Suge, H. and L. Rappaport. 1968. Role of gibberellins in stem elongation and flowering in radish. Plant Physiol. 43 : 1208-1214.

Zeevaart, J. A. D. 1983. Gibberellins and flowering. p. 333-374. In : A. Crozier (ed.). The biochemistry and physiology of gibberellins, Vol II. Praeger Scientific, New York.

Zenewich, K. P. and S. B. Rood. 1995. Vernalization and gibberellin physiology of winter canola. Endogenous gibberellin (GA) content and metabolism of [^{3}H] GA_1 and [^{3}H] GA_{20}. Plant Physiol. 108 : 615-621.

T. Nishijima, N. Katsura, M. Koshioka, H. Yamazaki, M. Nakayama, H. Yamane, I. Yamaguchi,
T. Yokota, N. Murofushi, N. Takahashi, M. Nonaka and L. N. Mander

低温および長日によるダイコンの抽台・花成における内生ジベレリンの役割

西島隆明[1]・桂　直樹[1,*]・腰岡政二[1]・山崎博子[1]・中山真義[2,**]・山根久和[2]・
山口五十麿[2]・横田孝雄[2,***]・室伏　旭[2]・高橋信孝[2,****]・野中瑞生[3]・Lewis N. Mander[4]

[1]農林水産省野菜・茶業試験場　514-2328　三重県安芸郡安濃町

[2]東京大学農学部　103-0032　東京都文京区

[3]農林水産省野菜・茶業試験場久留米支場　839-0851　福岡県久留米市御井町

[4]オーストラリア国立大学化学研究所　2601　オーストラリア　キャンベラ市

摘　要

低温および日長によるダイコンの内生ジベレリンの含量変化と，その抽台・花成との関係を調べた．ダイコンの茎葉部から，ジベレリン（GA）生合成経路の早期13位水酸化経路および早期非水酸化経路に属する18の内生GAを，ガスクロマトグラフ質量分析計（GC/MS）を用いて同定した．内生活性型GAと考えられるGA_1およびGA_4の茎における含量は，長日下および低温処理後の長日下で，短日下に比較して増加し，その増加は抽台・花成に先立っていた．低温処理およびその後の長日の，これら活性型GAの含量増加に及ぼす影響は相加的であった．これらの3β水酸化ジベレリン，つまりGA_1およびGA_4の，低温処理およびその後の長日による含量増加は，2β水酸化ジベレリンであるGA_{34}の含量低下を伴っていた．従って，低温処理およびその後の長日は，GA生合成経路における3β水酸化を活性化，2β水酸化を不活性化することが示唆された．以上のような，低温処理、ならびにその後の日長による内生GAの生合成の制御が，抽台・花成に関与すると考えられた．

*現在：農林水産省農業生物資源研究所
**現在：農林水産省野菜・茶業試験場
***現在：帝京大学バイオサイエンス学科
****現在：理化学研究所