Effects of Day Length and Air and Soil Temperatures on Sesamin and Sesamolin Contents of Sesame Seed

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Abstract: The lignans, e.g., sesamin and sesamolin, are components of the functional food sesame (Sesamum indicum L.) seed. This study aims to clarify the effects of environmental factors during ripening on the concentrations of these lignans to produce lignan-rich seeds. Here, we examined the effects of 4 factors (seeding time, day length, air and soil temperatures). The concentrations of sesamin and sesamolin in the seed from the capsule at different nodes on the stem were monitored using the high performance liquid chromatography (HPLC). A low air-temperature (22/15°C) during ripening increased the concentrations in the seed at the full-ripe stage of individual capsules compared with a high air-temperature (30/23°C). A short day-length (10-hr) and high soil-temperature did not affect the concentrations. The concentrations showed a tendency to increase with delay of seeding time. Under natural air-temperature conditions, the concentrations in the seeds from the capsules at a higher position on the stem were higher than those at a lower position, mainly due to the air temperature during ripening. The contents per seed were affected by the environmental factors through the difference in seed weight. The concentrations increased with the increase in seed dry weight and decreased with the desiccation of seeds during maturity. Under a low air-temperature condition, the rate of decrease in sesamin concentration was low, the accumulation period was longer and the maximum concentration of sesamolin was higher, resulting in higher contents of these lignans.

Key words: Day length, Lignan, Seed weight, Sesame, Sesamin, Sesamolin, Temperature.

The seeds and oil of sesame (Sesamum indicum L.) have been used for food around the world. The oil is characterized by an extraordinarily high stability. This appears to be related to antioxidant lignans contained in it, e.g., sesamin and sesamolin (Fukuda and Namiki, 1988). There is increasing interest in sesame as a functional food for improving human health, because sesamin and sesamolin have been reported to have various functions such as enhancement of liver metabolism and decrease in serum cholesterol level (Yamashita et al., 1992; Akimoto et al., 1993; Kang et al., 1998; Ashakumary et al., 1999). Therefore, the demand for the lignan-rich seeds is increasing. Recently, a new sesamin- and sesamolin-rich cultivar Gomazou was developed in Japan (Yasumoto et al., 2003), but it is still necessary to establish the cultivation technique to obtain lignan-rich seeds.

Sesame develops flowers at every axil on the stem above the first flowering node, and the flowers bloom in acropetal order along the stem. The number of flowering nodes on the main stem varied from 9 to 61 and the flowering duration varied from 16 to 55 d with seeding time (Kumazaki et al., 2002), suggesting that the environment during ripening varied with the node position of the capsule. Tashiro et al. (1991) reported that seed weight and sesamin and sesamolin contents vary with the node position of capsule on the stem. In that experiment, however, the age of harvested seeds differed with the node position of capsule on the stem, because all seeds in a plant were harvested at the time when the capsules on the lower position of the main stem turned yellow. Yasumoto et al. (2005) reported that sesamin and sesamolin contents of seeds changed with the days from flowering to harvest. Thus, it is assumed that the difference in the contents of sesamin and sesamolin among the seeds in the capsules at the different positions in a plant resulted from the differences in both the seed age at harvest and the environmental conditions during the ripening. This may be why the correlation of the contents of sesamin and sesamolin with seeding time was not clear in the study by Yasumoto et al. (2005), although the contents of sesamin and sesamolin varied with the seeding time.

The purpose of the present study is to clarify the effect of environmental factors, which vary with the seeding time, on the sesamin and sesamolin contents.
of seed to establish a cultivation technique for harvesting lignan-rich seeds. In the present study, the capsules at each node position were harvested at full-ripe stage in order to neglect the effect of seed age at harvest. First, we examined the effects of seeding dates and mulching on the contents of sesamin and sesamolin, because we previously observed that the mulching as well as the seeding time affects the growth, flowering and capsule markedly (Kumazaki et al., 2002). Second, we investigated the effects of environmental factors related to seeding time and mulching e.g., day length, and air and soil temperatures, on the contents of sesamin and sesamolin in seeds. Finally, we investigated the effect of air temperature on the change in contents of sesamin and sesamolin during ripening as compared with the change in seed weight.

Materials and Methods

1. Plant material and growth conditions

Sesame cultivar Masekin, which is a non-branching type cultivar with three capsules per leaf axil (a central capsule and two lateral capsules), was used in all experiments. Experiment 1 was conducted at the experimental field of Meijo University in Kasugai, Aichi. Experiment 2, 3, 4 and 5 were conducted at Meijo University in Nagoya, Aichi.

(1) Experiment 1

Seeds were sown on 22 May, 3 July and 14 Aug., 2001. At each seeding date, seeds were sown at 30 cm spacing on two ridges of 20 cm height and 3.9 m length with 100 cm spacing (13 plants per a ridge). Fertilizer which contained 15% N, 15% P₂O₅ and 10% K₂O was applied at a rate of 15.4 g per plant (7.5 g N m⁻²) as a uniform application. One ridge was mulched with black polyethylene film on the seeding date and named the mulched plots (MP). The other was not mulched and was called the non-mulched plots (NMP). One plant per hill was grown after thinning. The flowering date of central flower at all nodes on the main stem was recorded. The central capsules were harvested from 5 plants in each plot at the dehiscence time of individual capsules and were divided into groups of five consecutive nodes which were numbered acropetally; e.g., group 1 consisted of seeds at node position 1 to 5.

The data of air temperature was obtained from meteorological observation equipment in the experimental farm of Meijo University. Soil temperature at 15 cm depth of a ridge in MP and NMP without planting was measured every hour from 0900 to 1800 to determine the maximum soil temperature.

(2) Experiment 2

On 3 July, 2003, 4 seeds were sown at 4 spots in the central part of 10 pots (1/2000 a), each containing 10 kg soil. Fertilizer containing 15% N, 15% P₂O₅ and 10% K₂O was applied uniformly at a rate of 30 g per pot. The emerged seedlings were not thinned. Five pots were exposed to 10-hour day length every day after seeding by placing the pots in the dark from 1700−0700. This group was named the short-day (SD) group. The remaining five pots were exposed to natural day length as a control and named the natural day-length (ND) group. The natural day length decreased from 14.5 to 12.6 hours during the experimental period (from 3 July to 11 Sep.). Air temperature was not controlled. The central capsules were harvested from 5 plants in each group at the dehiscence time of individual capsules and were divided into the groups each at the same node position on the main stem.

(3) Experiment 3

On 19 June, 2005, the seeds were sown in 8 pots (1/5000 a), each containing 3 kg soil. Fertilizer containing 15% N, 15% P₂O₅ and 10% K₂O was
applied uniformly at a rate of 15 g per pot. The plants were thinned to one plant per pot, divided into two groups. One group was placed in a growth chamber (S-206W, Koito Industries, Ltd.) kept at 30/23°C (day/night, 0600-1800/1800-0600) and the other in a growth chamber (S-203W, Koito Industries, Ltd.) kept at 22/15°C after the first flowering day under natural daylight. The central capsules were harvested from 4 plants in each group at the dehiscence time of individual capsules and were divided into groups each at the same node position on the main stem.

(4) Experiment 4
On 8 May, 2004, the seeds were sown in 20 pots (1/2000 a), each containing 10 kg soil. Fertilizer containing 15% N, 15% P₂O₅ and 10% K₂O was applied uniformly at a rate of 30 g per pot. The plants were thinned to one plant per pot, divided into two groups. One group was exposed to a high soil temperature (HST) after seeding, using the heater wire (Nouden cable 100V 250W 31m, Tsukubadenki Co. Ltd.). The heater wire (156 cm per pot) was wound around a cylinder (16 cm diameter), and was formed into a spiral of 10 cm length. The spiral of heater wire was placed in each pot to produce a HST. The remaining 10 pots were exposed to natural soil temperature (NST) as a control. Air temperature was not controlled. The central capsules were harvested from 7 plants in each group at the dehiscence time of individual capsules and were divided into groups each at the same node position on the main stem.

(5) Experiment 5
The plants were grown as in experiment 3 using 20 pots (1/5000 a) in 2005. They were thinned to one plant per pot, divided into two groups, and grown at 30/23°C and 22/15°C after the first flowering day in the growth chambers. The flowering date of central flower at all nodes on the main stem was recorded. The central capsules were harvested from 10 plants in each group at 5-day intervals from 10 to 50 d after flowering (DAF) and at the time when capsule opened. At harvest, the seeds were detached from the capsule, their number per capsule was counted and their fresh weight was determined. Dry weight of seeds was measured after drying at 80°C for 48 hr.

2. Determination of sesamin and sesamolin contents of seed
Sesamin and sesamolin contents of seeds were determined according to the method of Shirato-Yasumoto et al. (2003). The harvested capsules were
dried in the room condition except the experiment 5; then seeds were collected from each capsule. The weight of 25 mature-seeds at each position on the main stem was measured. The seeds were homogenized with HISCOTRON (Microtech Nichion Co.) in 10 mL of 80% (v/v) ethanol for 30 seconds. The homogenate was centrifuged at 31000 × g (< 6ºC) for 6 minutes. The precipitate was homogenized in 10 mL of 80% (v/v) ethanol and centrifuged again. The first and the second supernatants were mixed and the mixture was used for HPLC analysis. Sesamin and sesamolin in the extract were separated by HPLC (PU-980, JASCO Co., Ltd.) with Nova-Pack C18 column (8×100 mm, Waters Co.) using 80% (v/v) methanol at a flow rate of 1.65 mL min⁻¹. They were detected at 290 nm of wavelength by UV detector (UV-970, JASCO Co., Ltd.) and their contents were determined from the peak areas at the retention time of around 3.5 and 4.5 minutes, respectively.

Results

1. Effect of seeding dates and mulching on the seed weight, and the sesamin and sesamolin contents of seeds

The plants seeded on 22 May started to bloom early in July and ended flowering late in Aug. or early in Sep. (Fig. 1). During the ripening period of the plants seeded on 22 May, the average day length was 13-hr and the average mean air temperature was 28ºC. The plants seeded on 3 July started to bloom early in Aug. and ended flowering in mid-Sep. In the plants seeded on 3 July, the day length during the ripening period was shorter and temperature was lower than those in the plants seeded on 22 May. The plants seeded on 14 Aug. started flowering in mid-Sep. and ended flowering early in Oct. During the ripening period of the plants seeded on 14 Aug., the day length was much shorter (11-hr day length on average) and air temperature was much lower (19ºC on average) as compared with the plants seeded on 22 May and 3 July. The plants with mulching (MP) started to bloom earlier than those without mulching (NMP) irrespective of seeding date, although the differences were small. The soil temperature during the ripening period in MP was 3−4ºC higher than that in NMP irrespective of seeding date, although the day length and air temperature in MP was almost the same as those in NMP.

The weight of seed from the capsules at the same position on the main stem increased with the delay of seeding date (Fig. 2A). The sesamin and sesamolin concentrations in the seeds also increased with the delay of seeding date (Fig. 2B, C), resulting in the increase in their contents per seed (Fig. 2D, E). There were little effects of mulching on the seed weight, concentration in the seed and content per seed of sesamin and sesamolin except in the plants seeded on 14 Aug.

The seed weight slightly decreased from the base to the top part along the main stem irrespective of
seeding date (Fig. 2A). The sesamin concentration in the seeds tended to increase from the base to the top part on the main stem, although the sesamolin concentration in the seeds was a little changed by the capsule positions (Fig. 2B, C). The change in the sesamin and sesamolin contents per seed was similar to that in their concentrations (Fig. 2D, E). However, the differences in their concentrations with the capsule positions were smaller than those in the concentrations.

The correlations between the mean air temperature during ripening and the concentration and the content per seed of sesamin and sesamolin were shown in Fig. 3, because the air temperature during ripening decreased with the delay of seeding date and the elevation of the capsule positions. The seed weight positively correlated with the temperature (Fig. 3A). Conversely, the concentration of sesamin and sesamolin showed the negative correlation with the temperature more clearly (Fig. 3B, C). The plants seeded on 14 Aug. had a smaller number of nodes with capsules as compared with those seeded on the other dates, and exhibited different changes in sesamin and sesamolin concentrations among the capsule positions (Fig. 2). As for the relationship between the concentrations and the air-temperature during ripening, however, the plants seeded on 14 Aug. was represented by the same regression line as the plants seeded on the other dates (Fig. 3). The content per seed of sesamin and sesamolin negatively correlated with the mean air temperature during the ripening period (Fig. 3D, E), because the decrease in their concentrations was larger than the increase in seed weight with the elevation of air temperature.

2. Effect of short-day treatment on the seed weight, and the sesamin and sesamolin contents of seeds

In this experiment, the average natural day length was 14.1 hr before flowering and 13.4 hr during the flowering period. The air temperature in the SD treatment from 1700−0700 was slightly higher than that in the ND treatment, although the difference was only 0.6ºC on the average. The average daily mean air temperature during the ripening period was 27.4ºC under the SD condition and 26.4ºC under the ND condition. The difference in mean air temperature during the ripening period was only 1ºC between the SD and ND conditions.

The seed weight was heavier in the SD group than in the ND group at every node-position on the main stem (Fig. 4A). The sesamin and sesamolin concentrations in seeds in the SD group were almost the same as those in ND group at the same node position, which bloomed at almost the same time (Fig. 4B, C). The sesamin and sesamolin contents per seed in the SD group were higher than those in ND group at the same node position (Fig. 4D, E).
The seed weight in both SD and ND groups decreased from the base to the top part along the main stem (Fig. 4A). The sesamin concentration in the seeds in both groups increased from the base to the top part on the main stem, although the sesamolin concentration was hardly changed by the node positions (Fig. 4B, C). The sesamin content per seed in both groups also increased from the base to the top part on the main stem (Fig. 4D). The sesamolin content in the SD group slightly decreased from the base to the top part, while that in the ND group did not vary with the node positions on the main stem (Fig. 4E).

The seed weight positively correlated with the mean air temperature during the ripening period, although the range of the temperature was narrow (Fig. 5A, 25.7−28.3°C). Conversely, the sesamin concentration in the seeds negatively correlated with the temperature (Fig. 5B). As a result, the sesamin content per seed showed no correlation with the temperature (Fig. 5D). The sesamolin concentration in the seed did not correlate with the air temperature (Fig. 5C). However, the sesamolin content per seed showed a positive correlation with the temperature (Fig. 5E), due to the increase in the seed weight with the elevation of the temperature (Fig. 5A).

3. Effect of air temperature on the seed weight, and sesamin and sesamolin contents of seeds

The soil temperature in the HST treatment was higher at 15 cm than at 2 cm in depth, although the temperature in the NST treatment was lower at 15 cm than at 2 cm in depth. The soil temperature at both depths in the HST treatment was higher than that in the NST treatment any time in the daytime (0800−1800 h). The difference in the soil temperature between the NST and HST treatment was larger at a 15 cm depth than a 2 cm depth. The soil temperature at 15 cm in depth in the HST treatment varied between 35 and 44°C, which was 7−15°C higher than that in the NST

4. Effect of soil temperature on the seed weight, and the sesamin and sesamolin contents of seeds

The soil temperature in the HST treatment was higher at 15 cm than at 2 cm in depth, although the temperature in the NST treatment was lower at 15 cm than at 2 cm in depth. The soil temperature at both depths in the HST treatment was higher than that in the NST treatment any time in the daytime (0800−1800 h). The difference in the soil temperature between the NST and HST treatment was larger at a 15 cm depth than a 2 cm depth. The soil temperature at 15 cm in depth in the HST treatment varied between 35 and 44°C, which was 7−15°C higher than that in the NST
Fig. 6. Effect of air temperature during the ripening period on one-seed weight (A), and the concentration in the seed (B, C) and content per seed (D, E) of sesamin and sesamolin in the capsules at different node positions on the main stem. The plants were grown at 30/23°C (closed square) and 22/15°C (open square) after the first flowering. Vertical bars in the figure indicate standard errors (n = 4).

Fig. 7. Effect of high soil temperature on one-seed weight (A), and the concentration in the seed (B, C) and content per seed (D, E) of sesamin and sesamolin in the capsules at different node positions on the main stem. The plants were grown at a high soil temperature (open triangle) and natural soil temperature (closed triangle) after seeding. Vertical bars in the figure indicate standard errors (n = 7).
treatment. The average of daily mean air temperature during the ripening period was 29.2°C under the both soil temperature conditions.

The seed in the HST treatment was heavier than that in the NST treatment at every node-position on the main stem (Fig. 7A). The seeds in the HST treatment contained the same levels of sesamin and sesamolin as those in the NST treatment at the same node-position. The sesamin and sesamolin contents per seed in the HST treatment were slightly higher than those in the NST treatment in many cases (Fig. 7D, E).

The seed weight decreased from the base to the top part along the main stem in both the HST and NST treatments (Fig. 7A). The sesamin and sesamolin concentrations in seeds did not significantly differ with the node position on the main stem at both soil temperatures, except that their concentrations in seeds from the top part was higher than those from the other parts (Fig. 7B, C). The variation of their contents per seed at different node positions were similar to that in their concentrations in seeds (Fig. 7D, E).

There was no correlation between the mean air temperature during ripening and the concentration of sesamin and sesamolin in the seeds, since the range of temperature was narrow (Fig. 8B, C, 28.8–29.5°C). The seed weight positively correlated with the temperature (Fig. 8A). Thus, the sesamin and sesamolin contents per seed showed positive correlation with the temperature (Fig. 8D, E).

5. Effect of air temperature on the accumulation of sesamin and sesamolin in the seed during ripening

The fresh weight of seed increased markedly until 10 d after flowering (DAF) and 15 DAF at 30/23°C and 22/15°C, respectively, then increased slowly until 30 and 40 DAF at 30/23°C and 22/15°C, respectively (Fig. 9A). Thereafter, the fresh weight decreased until the capsule opened at both temperatures. The maximum fresh-weight of seed at 22/15°C was much larger than that at 30/23°C. The capsule opened at 45 and 65 DAF at 30/23°C and 22/15°C, and the final fresh-weight of seed was heavier at 22/15°C than at 30/23°C.

The dry weight of seed increased until 30 DAF and 40 DAF at 30/23°C and 22/15°C, respectively (Fig. 9A). The increase at 22/15°C was slightly slower than that at 30/23°C. After that, the dry weight decreased slightly until the capsule opened at both temperature conditions. Both the maximum and final dry-weight of seed was heavier at 22/15°C than at 30/23°C.

The start of increase in the sesamin and sesamolin concentrations in seeds was later at 22/15°C (at 15−20 DAF) than at 30/23°C (at 10−15 DAF) (Fig. 9B, C). The concentrations increased with the increase in the dry-weight of seed at both temperatures. The increase in sesamin concentration was slightly slower at 22/15°C than at 30/23°C, although the increase in sesamolin
concentration was almost the same at the two temperature conditions. The sesamin and sesamolin concentrations reached a maximum at 20 DAF and 30−35 DAF at 30/23ºC and 22/15ºC, respectively. After that, the concentration decreased with the decrease in the dry weight. The decrease in sesamin concentration was markedly slower at 22/15ºC than at 30/23ºC, although the decrease in sesamolin concentration was almost the same at the two temperature conditions. As a result, the final sesamin concentration at 22/15ºC was higher than that at 30/23ºC, although the maximum concentration of sesamolin was lower at 22/15ºC. On the other hand, the final sesamolin concentration at 22/15ºC was higher than that at 30/23ºC as well as the maximum concentration.

Although the changes in sesamin and sesamolin contents per seed exhibited a pattern similar to those in the sesamin and sesamolin concentrations in the seed, the contents reached a maximum at 25−30 DAF and 35−40 DAF at 30/23ºC and 22/15ºC, respectively, which coincided with the time when the dry weight of seed reached the maximum (Fig. 9D, E).

**Discussion**

In the cultivation of sesame in the temperate regions, the environmental conditions during ripening period were extensively varied by seeding time and capsule position. Delay of seeding date and delay of flowering date with the elevation of node position shortened the day length and decreased the air and soil temperatures during the ripening (Fig. 1). Under field condition in experiment 1, the sesamin concentration in seeds increased from the base to the top part along the main stem under the natural air-temperature condition (Fig. 2), and was shown to negatively correlate with the air temperature during the ripening period irrespective of seeding date and mulching (Fig. 3). The results of pot experiments showed that the sesamin concentration was not changed by day length and soil temperature (Figs. 4, 5, 7, 8). It was shown that the seeds from capsules that ripened at high air-temperature contained a lower level of sesamin than those from capsules that ripened at low air-temperature in pot experiment under the constant air-temperature condition (Fig. 6). The concentration scarcely changed with the rise of capsule positions under the constant air-temperature condition (Fig. 6). Under the natural air-temperature condition in experiment 4, the change in sesamin concentration among the capsule positions was slight, because the fluctuation of mean air-temperature during ripening was very small (28.8−29.4ºC, Fig. 7). Thus, it seemed
that the variation in sesamin concentration among the seeding times and the capsule positions mainly resulted from the difference in air-temperature condition during ripening.

The sesaminol concentration in the seeds also tended to be higher at low air-temperature than at high air-temperature during the ripening period in experiment 1 and 3 (Figs. 2, 3, 6). However, this tendency was not so clear as that observed in the sesamin concentration. The sesaminol concentration was not changed by day length and soil temperature (Figs. 4, 5, 7, 8). Therefore, it was shown that effects of the environment during the ripening and the capsule position on the sesaminol concentration in seeds were small, although they showed the same tendency as the sesamin concentration.

In the present study, the seed weight increased with the elevation of air temperature during ripening (Figs. 3, 5, 8). Sesame growth is promoted by a temperature of 23−27°C (Weiss, 1983). Kumazaki et al. (2008) also showed that a high temperature of 30/23°C accelerated the stem elongation and the flowering as compared to a temperature of 22/15°C. In the range of air temperature during ripening of the present experiments, the elevation of air temperature seemed to promote the growth resulting in the increase in the seed weight. However, in experiment 3, the seed weight decreased with the elevation of the capsule position. Under the constant air-temperature condition, and the difference in the weight between 30/23°C and 22/15°C conditions was small. Thus, it seemed that the seed weight was also affected by the other environmental factors, e.g., the amount of solar radiation, because seeds in the capsules of a higher node position on the stem ripened under short day-length and low-intensity light conditions.

The sesamin and sesamolin contents per seed consist of their concentration in the seed and the seed weight. In experiment 2 and 4, the sesamin and sesamolin contents per seed correlated positively with the air temperature during ripening because the decrease in their concentrations was smaller than the increase in seed weight with increase in air temperature (Figs. 5, 8). On the other hand in experiment 1, the contents correlated negatively with the air temperature during ripening because the decrease in the concentrations with the increase in air temperature was larger than the increase in seed weight (Fig. 3). The yield of sesamin and sesamolin per seed, capsules or plant are sometimes important for agriculture, while the concentrations in them are important for eating oil. In this study, the contents per seed were changed complicatedly by environmental factors through the variation of seed weight, although the concentrations were simply affected by air temperature during ripening.

Sesame seeds showed an increase in fresh weight immediately after flowering, but the increase was due to water absorption and the increase in seed size (Fig. 9). The dry weight started to increase in the late period of fresh-weight increase. Both the fresh and the dry weights reached the maximum values at the same time. After that, the fresh weight decreased due to desiccation, and the dry weight also decreased slightly. The decrease in the dry weight seemed to result from the consumption by respiration during the active desiccation to maturity. The amount of sesamin and sesamolin in seed increased with the accumulation of dry matter and decreased with the seed desiccation immediately before the maturity. Therefore, the seed weight, the concentrations in the seed and contents per seed reached a maximum not at the full-ripe stage, but at the middle ripe stage. The decrease in the concentration and the content per seed of sesamin and sesaminol seemed to result from the change of the balance between synthesis of lignans and conversion to other lignans after the end of accumulation of dry matter (Jiao et al., 1998).

At a low air-temperature, sesamin accumulated slowly and the maximum concentration in the seed and maximum content per seed were lower than at a high air-temperature (Fig. 9). However, the concentration and content at dehiscence were higher at a low temperature because they decreased slowly. On the other hand, the sesaminol concentration in the seed and content per seed were higher at dehiscence at a low temperature, because the accumulation period was longer and the maximum concentration was higher at a low air temperature. Further study is needed to identify the cause of these differences between sesamin and sesaminol. In the study of Tashiro et al. (1991), in which all the seeds were harvested at the same time, the seeds from the capsules at upper nodes appeared to be closer to the stage at which the seed weight, the concentration and content per seed of sesamin and sesaminol reached a maximum, and appeared to ripen at a lower air-temperature than those from the capsules at lower nodes. This was considered to have resulted in higher concentrations of sesamin and sesaminol in the seeds from the capsules at upper nodes.

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* In Japanese with English abstract and summary.
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*** In Japanese.