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Dormancy in *Dioscorea*: Differences of Temperature Responses in Seed Germination among Six Japanese Species

NOBUO OKAGAMI AND MASASHI KAWAI

Biological Institute, Faculty of Science, Tohoku University, Sendai 980; Department of Biological Science, Faculty of Science, Hirosaki University, Hirosaki 036

Effects on seed germination of temperatures ranging from −2 C to +29 C were tested in *Dioscorea nipponica, D. tokoro, D. japonica, D. tenuipes, D. septemloba* and *D. quinqueloba* which orginate in the temperate zone; they are distributed from northern cold areas to southern warm areas approximately in this order in Japan. After water imbibition of these seeds, chilling induced full germination, and high temperatures over 23 C induced a secondary dormancy, but sensitivities to the chilling and to the high temperatures differed with species. Cold-climate species germinated rapidly at higher temperatures after a short-term chilling or even without chilling, whereas warm-climate species required chilling of a rather long period for germination; thus, among 6 species tested, favourable temperatures for germination and climatic temperatures of distribution area were conversely correlated.

Seeds of *D. tokoro* and *D. japonica* collected from several populations grown in different climates were also tested for germination at 11 to 29 C; seeds from warm climates germinated rather slowly compared to seeds from cold climates.

These inter- and intra-specific adaptation manners in the temperate members of the genus *Dioscorea* are entirely different from those of many other plant genera reported by some workers.

Key words: *Dioscorea* — Distribution — Dormancy — Seed germination — Temperature adaptation.

Research on the nature of various types of dormancy of many correlative species which belong to the same taxon and are distributed in different environments seems to give useful information about the process of adaptation to the environment. These comparative studies may supply some promising clues to various important problems in the physiological fields.

Dioscorea, the largest genus in Dioscoreaceae, consisting of about 200 species (Burkill, 1960; Ayensu, 1972), mainly grows in tropical Asia, Africa and America. In East Asia the genus is distributed continuously from the tropics to the cold temperate zone, and 14 species and 1 variety occur in Japan (Ohwi, 1953; Hatusima, 1971). Since Japanese species have different geographical distributions, their seeds may display different natures in regard to dormancy. The genus contains many bulbil-forming species (Dale, 1901; Plain and Burkill, 1936), and bulbils exhibit dormancy (Nakano and

Kinoshita, 1942; Okagami and Nagao, 1971). Therefore, comparison of the nature of dormancy between the bulbils and seeds is very interesting in relation to diversity of dispersion manner of the genus.

For these reasons, we have been studying several physiological natures of dormancy of seeds and bulbils of *Dioscorea* (Okagami and Nagao, 1971; Okagami and Kawai, 1977; Okagami, 1978, 1979). In the present study, seeds of Japanese *Dioscorea* species which have different geographical distributions were tested for their responses in germination to various temperatures and to light. Thereby we considered some dormant natures common in the genus and specific to individual species. Previously, Iwao (1980) observed the seed germination of cultured plants of several *Dioscorea* and considered the relationship between flowering time and germination habits. To the contrary, our work was performed to obtain a relationship between geographical distribution and germination, and a peculiar adaptation manner towards temperatures was observed.

Materials and Methods

Plant materials

Seeds of 6 *Dioscorea* species tested were collected at the natural habitats listed in Table 1 and marked in Fig. 1. For each species 30–40 mother plants which grew within circle of about 1 km in radius served as specimens. Eight other species and 1 variety could not be used in the present work because they have limited or low-density distribution areas, or because very few female plants occur in Japan.

The seeds were collected on 28 Oct.–1 Nov. 1976, 17–22 Oct. 1977, 26–30 Oct. 1978 and 30 Oct.–4 Nov. 1979, at which times the seeds reached full maturity and were ready to shed. After collection, seeds were stored in a desiccator with silica gel at room temperature in the dark for about 1 month, until use. Under these conditions seeds retained the germinability unaltered for about 1 year.

Table 1. Geographical sources of materials used

Species	Localities[1)]
Dioscorea nipponica Makino	Niigata
D. tokoro Makino	Kagoshima, Mie, Miyagi, Akita
D. japonica Thunb.	Kagoshima, Mie
D. tenuipes Franch. et Savat.	Kagoshima
D. quinqueloba Thunb.	Kagoshima
D. septemloba Thunb.	Niigata

[1)] See legend to Fig. 1.

Germination test

To examine the effects of various temperatures on seed germination 12 incubators were set at temperatures ranging from –2 C to +29 C at 2–3 C intervals. As described in a previous paper (Okagami and Kawai, 1977), 40–70 seeds were placed in a 9-cm Petri dish on a thin layer of absorbent cotton moistened with distilled water and allowed to stand in an incubator with or without illumination (2.1 W/cm^2) by white light from

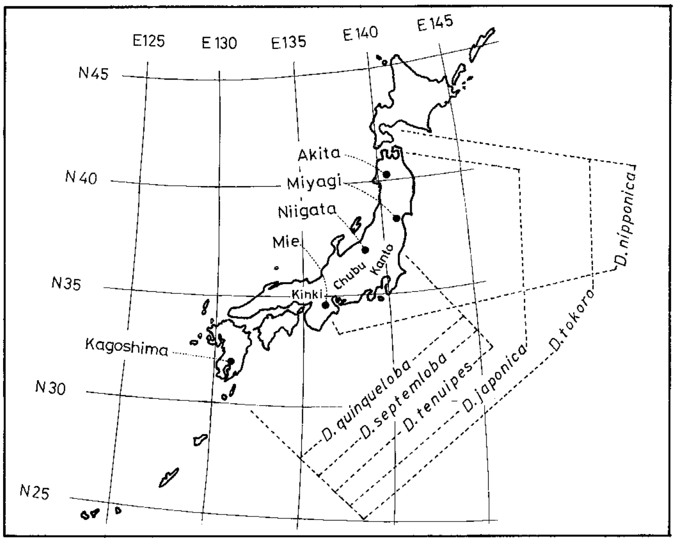

Fig. 1. Places of seed collection and approximate distribution area of the species used. Akita: Tashiro, Kita-Akita, Akita Pref.; Miyagi: Aobayama Botanical Garden, Tohoku Univ., Sendai, Miyagi Pref.; Niigata: Yagisawa, Minami-Uonuma, Niigata Pref.; Mie: Tsuge, Iga, Mie Pref.; Kagoshima: Ohkubo, Aira, Kagoshima Pref. Dotted lines with species names indicate approximate limits of their distribution area as judged from the following articles; Ohwi (1953), Hatusima (1961, 1971), Takeuchi *et al.* (1970), Takeuchi (personal communication), Okuyama (1977). The precise limits and range of most of the species being indefineable in simple terms, the above illustrations are intended as an exemplification of the distribution. Though *D. tenuipes*, *D. septemloba* and *D. quinqueloba* have the same northern and southern limits of distribution area as shown in the figure, *D. tenuipes* grows frequently in Kanto district and southwards, *D. septemloba* in the Chubu district and southwards and *D. quinqueloba* in the Kinki district and southwards.

fluorescent lamps (Real Daylight, 40-D-SDL, Toshiba, Tokyo). The air temperatures of the incubators were controlled within ± 0.6 C, and fluctuations in temperature of the seeds were within ± 0.1 C. Germination was counted under the light of a green or dim white fluorescent lamp and scored as the time of radicle tip protrusion through the seedcoat.

Results

Germination ability under various temperature conditions

Effects on germination of temperatures ranging from 11 C to 29 C were examined in the light or in the dark for 20 and 70 days (Fig. 2). Within 70 days, germination percentages of all lots reached plateaus (e.g. Fig. 3). *D. nipponica* seeds germinated at about 23–26 C within 20 days and increasingly germinated at higher temperatures during an additional 50 days, whereas the other species could not germinate practically at the temperatures above 23 C. *D. japonica* and *D. quinqueloba* seeds did not germinate within 20 days, but did germinate during an additional 50 days. *D. septemloba* seeds completely failed to germinate within 70 days; they did not germinate

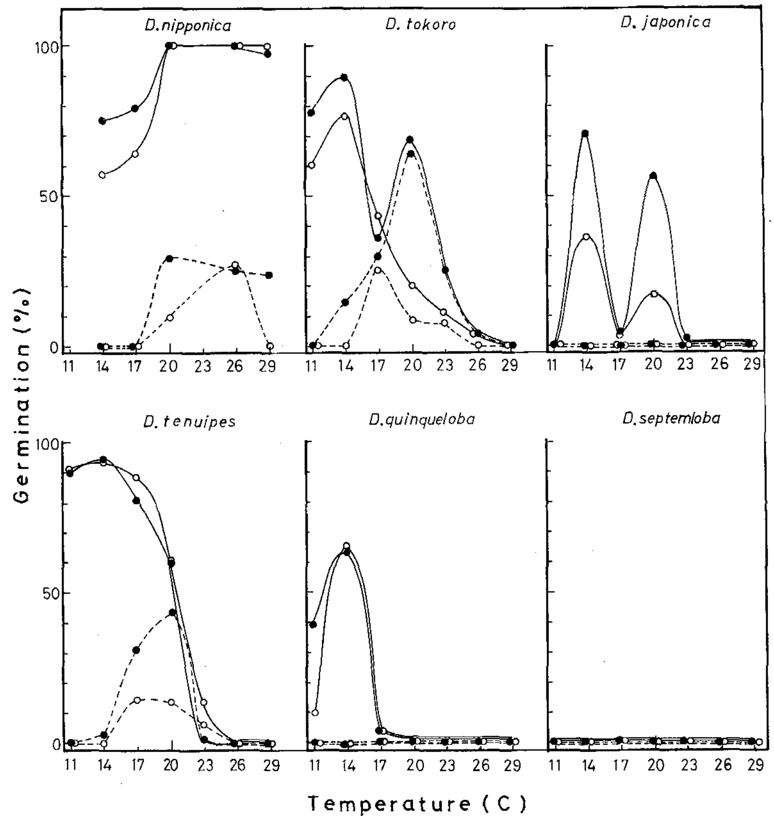

Fig. 2. Germination at various temperatures of six species of Japanese *Dioscorea* seeds. The seeds were incubated at various temperatures in the light (open circles) or in the dark (closed circles) for 20 days (dotted lines) or 70 days (solid lines). The seeds used were collected from Kagoshima (*D. tokoro, D. japonica, D. tenuipes, D. quinqueloba*) and from Niigata (*D. nipponica, D. septemloba*) in 1976.

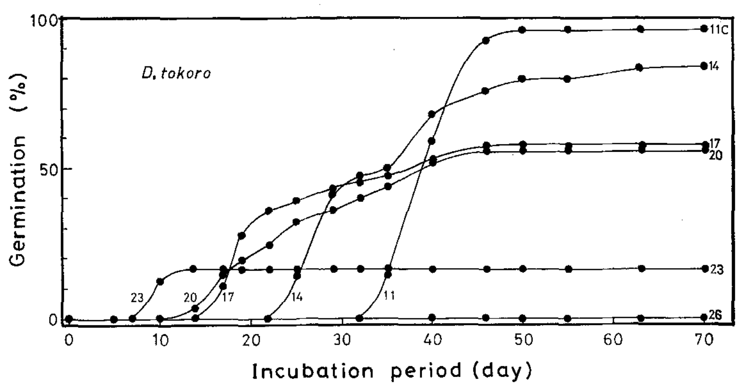

Fig. 3. Time courses of germination of *D. tokoro* seeds at various temperatures in the dark. The seeds collected from Kagoshima in 1978 were used. The numerals in the figure indicate the incubation temperature (C).

even when the incubation period was extended to 300 days (data not shown). The optimum temperature for the germination of *D. tokoro* and *D. tenuipes* seeds was 20 C at the 20th day and was 14 C at the 70th day; namely germination started rapidly at higher temperatures and slowly at lower temperatures, but the final germination percentages were higher in the latter. Time courses of germination at various temperatures in the case of *D. tokoro* are shown in Fig. 3.

The lower germination abilities of *D. tokoro* and *D. japonica* at 17 C gave double optimum curves (Fig. 2). This phenomenon was observed occasionally but not usually; it depended on the year in which the seeds were collected (e.g. Fig. 4).

Irradiation with white light usually inhibited germination (Fig. 2), and the degree of the inhibition differed with species, and with the temperature and period of incubation.

Differences in germination ability among seeds collected in different years

To examine differences in germination abilities among seeds collected in different years, the seeds collected in 1977, 1978 and 1979 were incubated at various temperatures

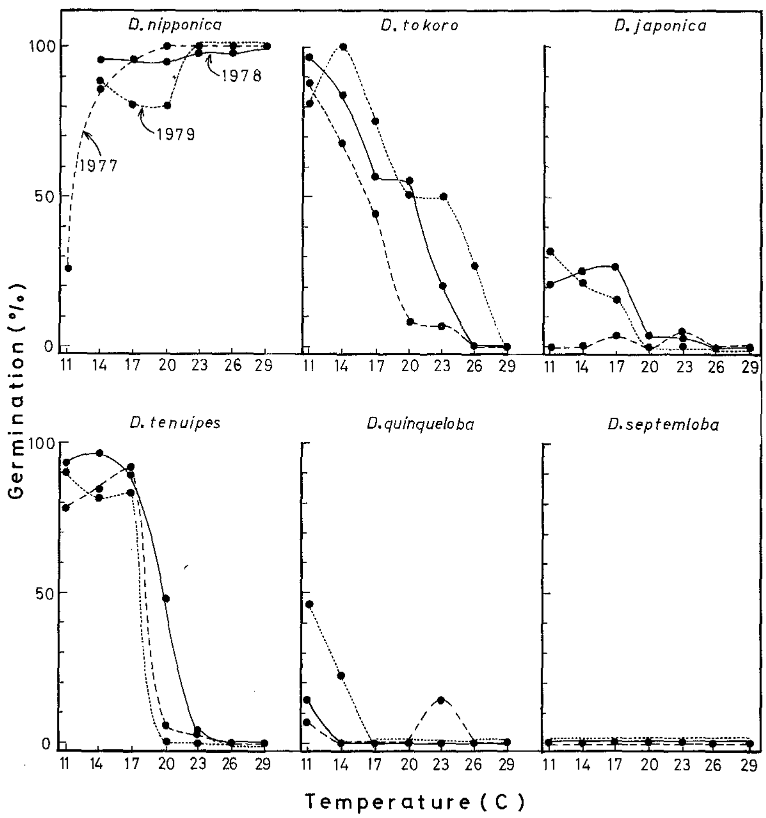

Fig. 4. Germination at various temperatures of *Dioscorea* seeds collected in 1977 (broken lines), 1978 (solid lines) and 1979 (dotted lines). The seeds were incubated in the dark for 70 days. Places of seed collection were the same as described in Fig. 2.

about 1 month after the collection in each year (Fig. 4). Though the germination percentages of the seeds of each species at the respective temperatures varied with the year of collection, characteristic differences in germination responses among the tested species were observed in seeds collected in 1977–1979 (Fig. 4) as observed in seeds collected in 1976 (Fig. 2).

Dormancy break by chilling

Effective chilling temperatures. As reported previously, chilling potentiates germination of *D. tokoro* and *D. tenuipes* seeds (Okagami and Kawai, 1977). To find the temperatures favorable for dormancy break, seeds of 6 species were chilled at various temperatures and then incubated at 20 C for germination (Fig. 5). Optimal temperatures for dormancy break were 5 C in *D. tokoro, D. tenuipes* and *D. quinqueloba,* and 0 C for *D. japonica* and *D. septemloba. D. nipponica* was capable of germinating without chilling (Fig. 2 and 4), but its germination was slightly promoted by a

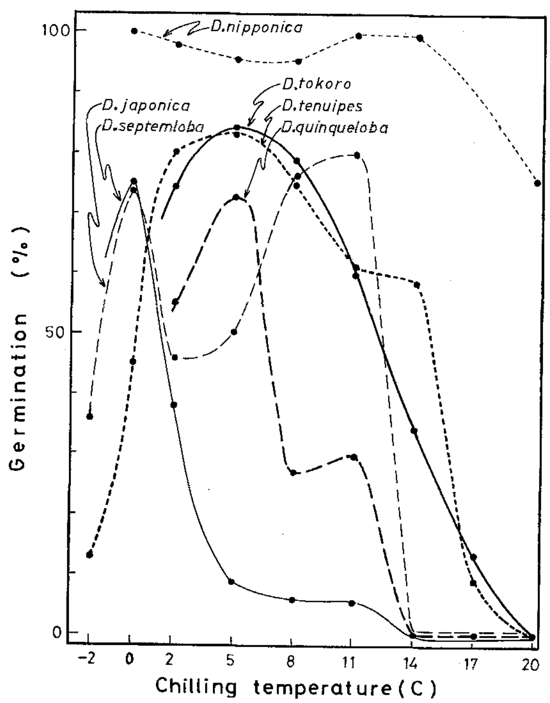

Fig. 5. Effect of chilling temperatures on dormancy breaking in *Dioscorea* seeds. The seeds were treated at various temperatures below 20 C in the dark, and then allowed to germinate at 20 C in the dark. Durations of the chilling were 30 days in *D. nipponica* and *D. tenuipes,* 20 days in *D. tokoro,* 100 days in *D. japonica,* 75 days in *D. quinqueloba* and 65 days in *D. septemloba.* Duration of the incubation at 20 C after the chilling were 30 days in *D. nipponica* and *D. tenuipes,* 15 days in *D. tokoro* and *D. quinqueloba,* 40 days in *D. japonica* and 60 days in *D. septemloba.* The seeds used were collected from Kagoshima and Niigata in 1976. Data do not include the counts of seeds which germinated during the chilling periods.

chilling pretreatment (Fig. 5). *D. japonica* seeds treated at 8 and 11 C germinated to almost the same degree as those chilled at 0 C, but the rate of shoot elongation was higher in the seedlings from the latter than in those from the former. The optimum temperatures for dormancy break were unaltered with the collection year of the seeds (data not shown). Immature seeds of several species also required chilling, but at slightly higher temperatures than required by the mature seeds, for breaking dormancy (data not shown).

Shift of germination temperatures after the chilling. To examine whether or not the chilling pretreatment altered the temperature dependency of seeds in germination, chilled seeds were incubated at various temperatures in the light or in the dark (Fig. 6). The chilled seeds were capable of germinating at higher temperatures (17–26 C) unfavorable for germination of the unchilled seeds (Fig. 2 and 4) except *D. nipponica* seeds which germinated at such temperatures without the chilling.

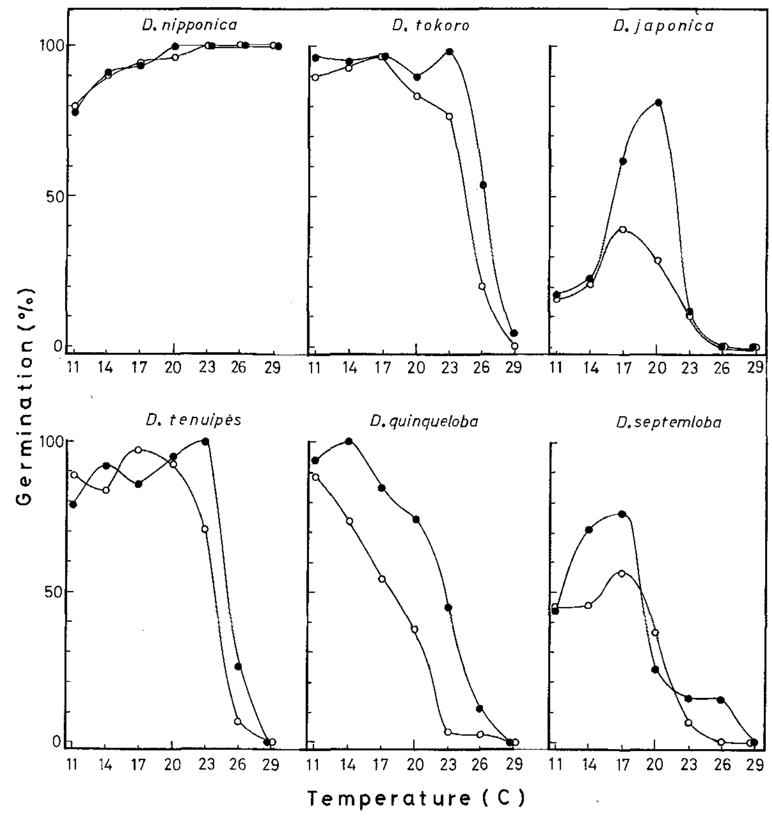

Fig. 6. Germination at various temperatures of *Dioscorea* seeds after chilling. The seeds were chilled at 5 C (*D. nipponica, D. tokoro, D. tenuipes, D. quinqueloba*) or at 0 C (*D. japonica, D. septemloba*) and then incubated at various temperatures in the light (open circles) or in the dark (closed circles). *D. nipponica, D. tokoro* and *D. tenuipes* seeds were chilled for 35 days and incubated for 50 days. *D. japonica, D. quinqueloba* and *D. septemloba* seeds were chilled for 65 days and incubated for 85 days. The seeds used were collected from Kagoshima and Niigata in 1978.

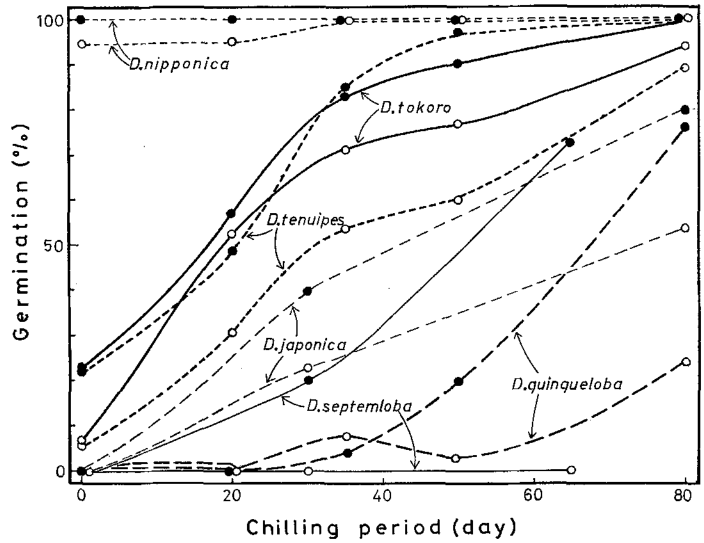

Fig. 7. Germination abilities of *Dioscorea* seeds after various periods of chilling. The seeds were chilled in the dark at 5 C (*D. nipponica, D. tokoro, D. tenuipes, D. quinqueloba*) or at 0 C (*D. japonica, D. septemloba*) for various periods as indicated in the abscissa. Then the chilled seeds were allowed to germinate at 20 C in the light (open circles) or in the dark (closed circles). Germination was counted after 100 days from the start of the chilling. The seeds used were collected from Kagoshima and Niigata in 1977.

Chilling periods necessary for dormancy break. The seeds of 6 species were chilled for various periods and then were incubated at 20 C in the light or in the dark (Fig. 7). *D. nipponica* seeds germinated actively almost irrespective of the chilling periods. *D. tokoro* and *D. tenuipes* germinated fully even after short-term chilling as reported previously (Okagami and Kawai, 1977). On the other hand, *D. japonica, D. quinqueloba* and *D. septemloba* required more prolonged chilling periods for rapid germination.

White light irradiation during the incubation for germination after the chilling inhibited the germination. The inhibition of germination decreased with an increase of the chilling periods except in the case of *D. septemloba*; in *D. septemloba* the inhibition was very strong even after the prolonged chilling.

Differences of germination abilities of D. tokoro *and* D. japonica *seeds collected at different places*

D. tokoro seeds harvested from 4 prefectures, Kagoshima, Mie, Miyagi and Akita, were incubated at various temperatures without chilling (Fig. 8). The seeds collected at northern cold places (Akita and Miyagi) germinated more rapidly than the seeds collected at middle (Mie) and southern (Kagoshima) warm places. This tendency was observed in both 1977 and 1978, though germination percentages during a definite incubation period differed with year. The tendency observed in *D. tokoro* seeds was also observed in *D. japonica* seeds collected in 1978; the seeds from the Mie (middle)

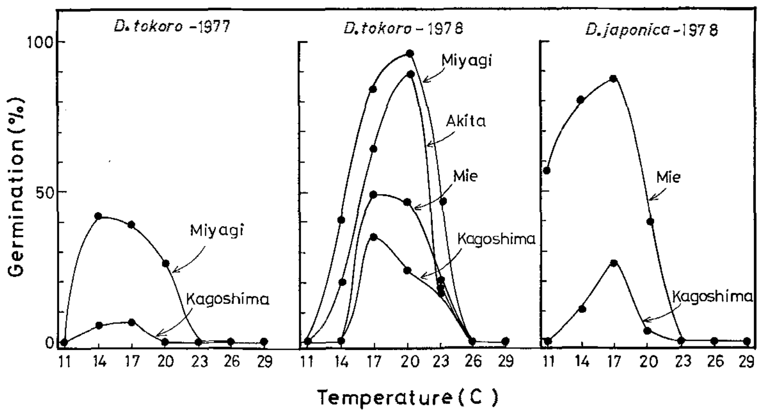

Fig. 8. Germination of *D. tokoro* and *D. japonica* seeds collected at various locations. The places and years in which the seeds were collected are indicated in the figure. The seeds of *D. tokoro* were incubated at various temperatures in the dark for 25 and 22 days in 1977 and 1978, respectively, and those of *D. japonica* were similarly incubated for 45 days.

population germinated more rapidly than the seeds from the Kagoshima (southern) population.

Discussion

As reported here, in seeds of the 6 species used (1) chilling broke dormancy, (2) high temperatures inhibited germination and (3) white light irradiation inhibited germination. The seeds of all the species used had these three properties in common but to different extents.

The seeds of *Dioscorea* showed the chilling requirement for the breaking of dormancy as did the bulbils of *Dioscorea* (Okagami and Nagao, 1971; Okagami, 1979). However, the optimum chilling temperature in the seeds differed from species to species (0 or 5 C, Fig. 5), whereas in bulbils it was almost the same, i.e. 5 C, for all species tested including *D. japonica* (Tanno and Okagami, 1974). The differences in the optimum temperatures between seeds (0 C) and bulbils (5 C) of *D. japonica* suggest that the adaptation mechanism may be different between the sexual and asexual dispersal organs of this species.

The chilling treatment reversed the inhibition caused by a high temperature treatment as it broke the natural dormancy of the seeds (Kawai and Okagami, unpublished data). The germination inhibition by high temperature is, therefore, considered as a secondary dormancy. The high-temperature-induced dormancy is characteristic of the seeds, since in bulbils no sprouting inhibition by high temperatures was observed (unpublished data).

Detailed analysis on the relationship between seed germination habits (Fig. 2, 4 and 7) and the geographical distributions (Fig. 1) of Japanese *Dioscorea* species revealed that there exists the tendency for species which are distributed in the northern locations to germinate rapidly at rather higher temperatures and require the chilling to a lesser extent for dormancy break. Exceptionally, *D. japonica* seeds germinated poorly at higher temperatures and required more chilling than *D. tenuipes* seeds, whereas the northern limit of distribution of *D. japonica* lies north of that of *D. tenuipes*. This discrepancy may be ascribable to the taxonomical position of *D. japonica*; *D. japonica* belongs to section *Enantiophyllum*, while the other 5 species belong to section *Stenophora* (Burkill, 1960). From a different viewpoint, the discrepancy may be attributable to the characteristic dispersion manner of *D japonica*. Among the 6 species used, *D. japonica* is the only species which forms both seeds and bulbils; and its seed productivity is very poor in the northern half of its distribution area; moreover, seeds of this species germinate belatedly in cold places whereas production and sprouting of bulbils do not differ with habitat (unpublished data). Therefore it is conceivable that *D. japonica* disperses by bulbils all over its distribution area, but by seeds as well as bulbils in the southern half of its distribution area. Probably the northern limit of the latter distribution area of *D. japonica* lies almost parallel to or south of that of *D. tenuipes*. If this assumption is correct, the germination habits of *D. japonica* seeds are not discrepant to the above-stated general rule on temperature responses and habitats.

A tendency similar to that postulated above is observed in intraspecific populations of *D. tokoro* and of *D. japonica*; without chilling treatment seeds from northern populations germinated more rapidly than did seeds of southern populations (Fig. 8).

On the contrary, in European and North American plant genera, without exception, the seeds produced in warm climates required less chilling and germinated more quickly at rather broader temperatures than did those in colder climates (Haasis and Thrupp, 1931; Sterns and Olson, 1958; McNaughton, 1966; Thompson, 1968, 1970; Nikolaeva, 1969). With Asian plants, however, no report is available, except ours, dealing with the relationship between their temperature dependent germination habits and natural geographical distribution. Whether the unusual adaptation manner of *Dioscorea* seeds to temperatures is common to other temperate plant genera of East Asia or not is a very interesting but yet unanswered question.

Seed germination involves highly integrated physiological processes and our experimental conditions are different from natural conditions; however, the germination habits described in the present paper were also observed when the seeds were sown under natural conditions in Sendai (data not shown). It may be possible to assume that the seeds of species and populations of northern distributions are in an imposed dormancy while the seeds of southern species and populations are in an innate dormancy.

A preliminary experiment for observation of an alteration of the dormant state during the maturation of *Dioscorea* seeds showed an interesting dormant state of immature seeds. The seeds of *D. quinqueloba*, a southern species (Fig. 1), reached the deepest dormant state when completely matured, whereas the seeds of northern species such as *D. nipponica*, passed through their deepest dormant state at an immature stage and stayed at an almost awakened state when matured. These findings indicate a possible physiological process of adaptation of this genus to temperature conditions of the temperate zone of East Asia. It is very interesting to observe and to compare ontogenetic alterations of dormant characters of seeds of *Dioscorea* distributed in the tropics, subtropics and temperate zones.

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