Prolongation of seed dormancy by dry storage in *Brassica japonica* SIEB.

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Summary

The seeds of "Mizuna," *Brassica japonica* SIEB., usually have dormancy during two months after harvest, but, when they are stored in the desiccator, the dormancy continues for as long as more than three years. The dormancy of such desiccated seeds is partial or incomplete, and their percentage germination shows a considerable fluctuation during the period of prolonged dormancy. When desiccated, not only mature seeds but also immature ones show prolongation of dormancy, and the former seems to be less dormant than the latter. When dormant seeds are taken out from the desiccator, their dormancy is completely removed in four weeks, but non-dormant seeds do not enter dormant state even if they are put into the desiccator. In germination, desiccated seeds are more sensitive to temperature than air-dry seeds. Following methods are partially useful for breaking dormancy, i.e., low-temperature, slitting or removing of the seed coat, and soaking in running water.

Introduction

It is recognized that the seeds of *Cruciferous* vegetables have dormancy during a certain period after harvest(2,6,11,12,13) and that the length of dormancy varies with species or cultivars ranging from 0 to 140 days(13). As to *Brassica japonica* SIEB., "Mizuna," which is locally cultivated in some parts of Japan, its dormancy was reported to continue for 70 days after harvest(13). In *Cruciferous* seeds, the effect of some environmental factors upon germination, such as temperature, light and oxygen pressure, was examined(3,4,9). And various treatments such as low-temperature, slit seed coat, excised embryo, absolute alcohol, ether, concentrated sulphuric acid, boiling water, running water, thiourea, etc. were tried as methods for breaking dormancy of seeds(2,4,12). On the other hand, there are no reports about the effect of storage condition upon dormancy of seeds, especially about the relation between desiccation of seeds and their dormancy.

This paper concerns a peculiar phenomenon of *Brassica japonica* seeds, i.e., prolongation of dormancy caused by dry storage, as well as some methods for breaking dormancy.

Methods and results

1. Prolongation of seed dormancy by desiccation

Open-pollinated mature seeds collected from the experimental farm on the 20th of May in 1966 were divided into two groups, one of which was stored into the desiccator containing silica gel and the other was left on the desk in the laboratory and air-dried. Then 50 seeds were taken out every 10 days from each group to test their germination until 50 days, but afterwards tests were made at irregular intervals. Seeds were laid on two sheets of blotting paper moistened by water in petri dishes, and the number of germinated seeds was counted every day during a week. The tests were carried out at room temperature. "Percentage

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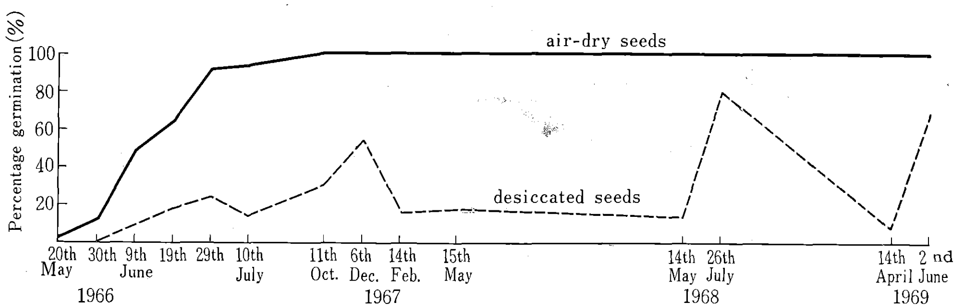

Fig. 1. Transition of percentage germination of *B. japonica* seeds during the period of prolonged dormancy by storage in the desiccator.

germination" was calculated by the sum total of seeds which germinated during 7 days. The results are shown in Fig. 1. As seen in Fig. 1, the dormancy of air-dry seeds on the desk gradually disappeared and their germination amounted to 100% in about two months after harvest, while the seeds stored in the desiccator continued to be partially dormant at least during more than three years and their germination showed a considerable fluctuation during this period.

2. Maturity of seeds and dormancy

Seeds at different stages of development were harvested on the 9th, 14th, 20th, 24th and 29th of May in 1968, respectively, and stored in the desiccator immediately after harvesting. The seeds collected on the 9th and 14th were green and immature, but the seeds of the 20th were yellow and those of the 24th and 29th were brown and mature. Germination tests were made every 5 days, but, afterwards, every 10 or 15 days. Fig. 2 presents the effect of desiccation upon seeds with different maturities. In the most immature seeds from the 9th, there was no difference in percentage germination between air-dry seeds and desiccated ones and more than 80% of them were non-germinative perhaps not due to dormancy but due to undeveloped state. As to the immature seeds from the 14th, the percentage germination of air-dry seeds remained 80% at maximum and that of desiccated seeds did not exceed 15%. The reason why air-dry seeds did not reach 100% seems to be ascribable to the fact that this population contained some green seeds which might be too young to germinate. In the mature seeds harvested from the 20th through the 29th, the dormancy of air-dry seeds disappeared in one and a half months and the maxima of their germination were reached coincidently on the 8th of July. While, desiccated mature seeds were partially dormant during more than one year, among which the percentage of non-dormant seeds became higher as seeds were harvested later. Thus not only mature seeds but also immature ones showed prolonged dormancy by dry storage, and, moreover, the dormancy of the latter seemed to be deeper than that of the former. The ratios of the germination of desiccated seeds to that of air-dry seeds from the 14th, 20th, 24th and 29th of May were 5.9%, 10.2%, 13.4%, and 19.0% on the average, respectively.

3. Change of dormancy in seeds transferred to different condition

As materials, the seeds harvested on the 20th of May in 1966 (cf. Fig. 1) were used. On the 11th of October in the same year, a sample of the seeds which had been stored in the

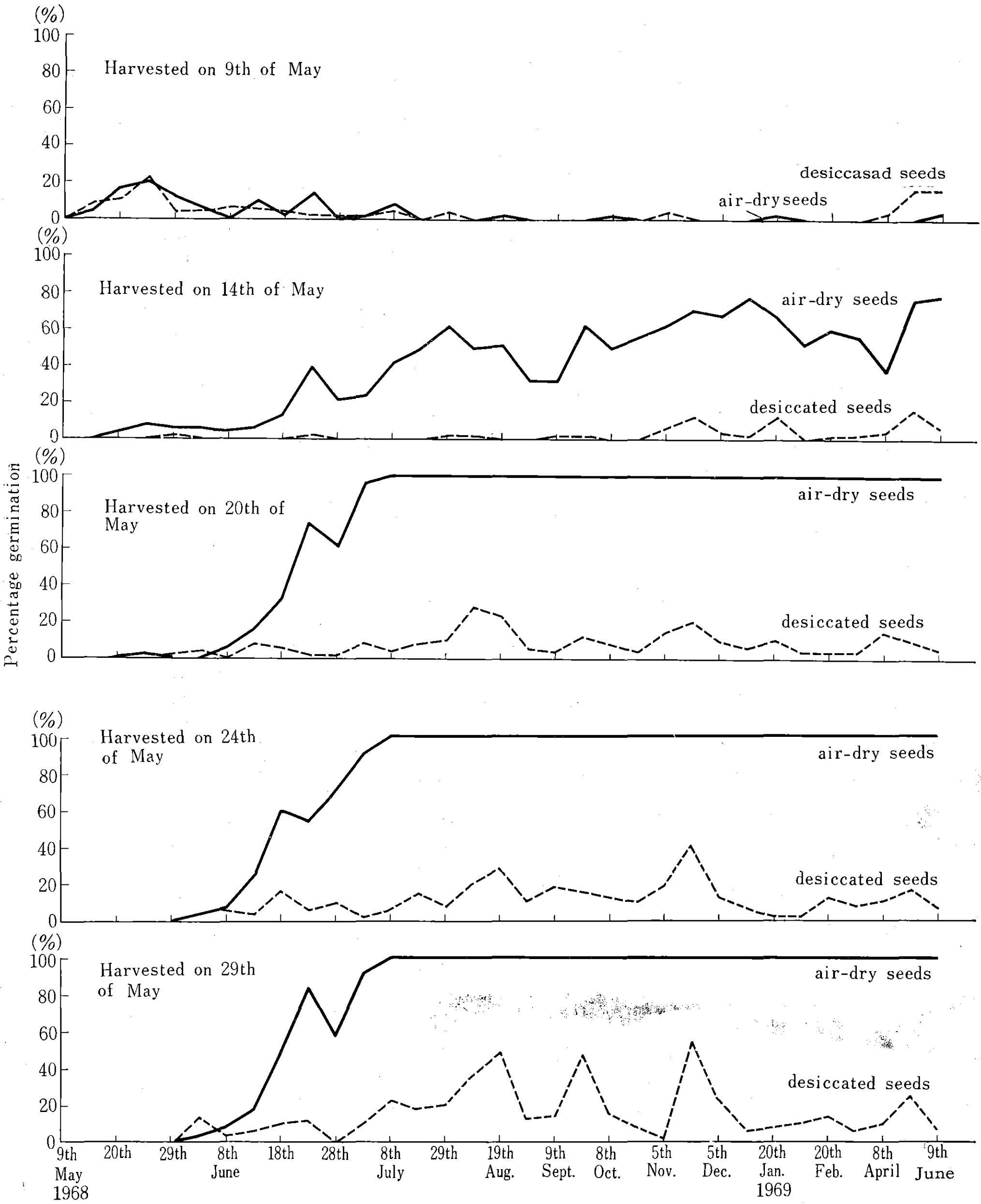

Fig. 2. Relation between the maturity of seeds and prolonged dormancy by storage in the desiccator.

desiccator were transferred to the room and, conversely, some of the seeds which had been left on the desk were put into the desiccator. After that, similar procedures were repeated 8 times at intervals of one week. All samples of seeds transferred at different dates were tested simultaneously on the 6th of December. The results are shown in Table 1. When dormant seeds were taken out from the desiccator, their dormancy was completely removed in four weeks, but non-dormant seeds did not enter dormant state even if they were put into the

Table 1. Removal of dormancy of seeds transferred from the desiccator to the room, and *vice versa*.

Date of transference of seeds	Days before germination test (week)	Number of seeds	Number of germinated seeds							Date when the germination of 50 seeds was completed
			Dec. 7 th	8 th	9 th	10 th	11 th	12 th	Total	
From desiccator to room 1966										
Oct. 11	8	50	18	32	—	—	—	—	50	
Oct. 18	7	50	21	29	—	—	—	—	50	
Oct. 25	6	50	15	33	2	—	—	—	50	
Nov. 2	5	50	5	33	10	2	—	—	50	1967
Nov. 8	4	50	6	30	10	3	0	0	49	Feb. 20
Nov. 15	3	50	6	17	11	4	2	1	41	July 9
Nov. 22	2	50	3	19	5	8	3	3	41	July 24
Nov. 30	1	50	6	16	2	2	2	1	29	July 24
Dec. 6	0	50	2	16	3	1	3	2	27	Sept. 27
From room to desiccator										
Oct. 11	8	50	0	47	3	—	—	—	50	
Oct. 18	7	50	0	50	—	—	—	—	50	
Oct. 25	6	50	0	47	1	1	1	—	50	
Nov. 2	5	50	0	48	2	—	—	—	50	
Nov. 8	4	50	0	49	1	—	—	—	50	
Nov. 15	3	50	0	48	2	—	—	—	50	
Nov. 22	2	50	0	48	2	—	—	—	50	
Nov. 30	1	50	0	49	0	0	1	—	50	
Dec. 6	0	50	0	50	—	—	—	—	50	

desiccator. The data in Table 1 are the results up to 7 days after placing seeds on moist blotting paper, but this experiment was extended to 9 months, during which attention was paid to keeping the seeds in moist condition. It was found during this period that a few seeds were constantly germinating in a sporadic manner. As seen in Table 1, it was in September next year when all seeds which remained dormant finished germination.

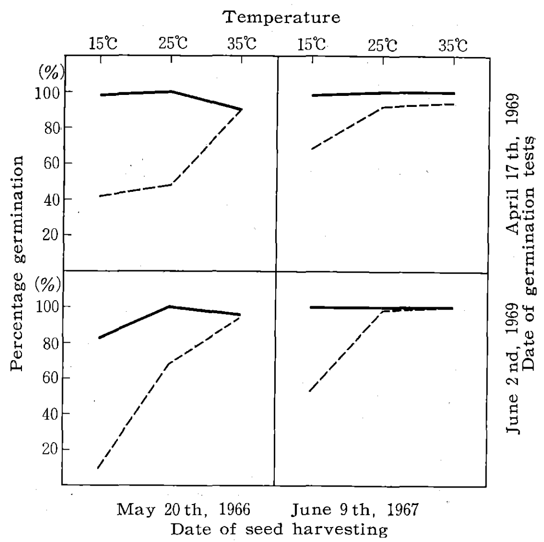

Fig. 3. Relation between temperature and percentage germination of air-dry seeds as well as desiccated ones. Air-dry seeds ——; Desiccated seeds ----

4. Temperature and the percentage germination of seeds

Germination tests of the seeds harvested in May of 1966 (cf. Fig. 1) and in June of 1967 were made at 15°C, 25°C and 35°C in thermostats in April and June in 1969, respectively. The results are shown in Fig. 3. Air-dry seeds showed a fairly good germination in every case and at 25°C always the best. While, the percentage germination of desiccated seeds varied widely with different temperatures, and it showed the maximum at 35°C and then decreased according as the temperature became low. The optimum temperature of the air-dry seeds is not the same as that of the desiccated ones.

It may be said that the desiccated seeds are more sensitive to temperature than the air-dry seeds. After the tests on June the 2 nd were finished, ungerminated seeds remaining in petri dishes at various temperatures were collected, their seed coats were removed, and bare embryos were soaked in running water. As a result, they all germinated and proved to be living.

5. **Methods for breaking dormancy**

(a) **Low-temperature treatment** The seeds, which were harvested on the 12 th of May in 1967 and stored in the desiccator, were placed on moist paper in petri dishes on the 21 st of June. Then they were put into the refrigerator at about 5°C during 48 hours and transferred to the room on the 24 th of June. The germination of air-dry seeds as well as desiccated ones, both of which were not exposed to low-temperature, was also tested. The result is presented in Table 2. In this case, not only mature seeds collected from ripe capsules but immature seeds from unripe capsules were used. In the air-dry condition, the removal of dormancy in mature seeds was complete, but that in immature ones was partial or incomplete. Similarly the dormancy of desiccated seeds was deeper in immature state than in mature one. Low-temperature treatment could partially break the dormancy of desiccated seeds, and immature seeds were more strongly affected by temperature than mature ones.

Table 2. Effect of low-temperature treatment on the breaking of dormancy in *B. japonica* seeds.

Maturity of seeds	Storage condition	Treatment	Number of seeds	Percentage germination (%)
Mature	Air-dry	Untreated	50	100
	Desiccated	Untreated	50	50
	Desiccated	Low-temperature	50	66
Immature	Air-dry	Untreated	50	88
	Desiccated	Untreated	50	24
	Desiccated	Low-temperature	50	50

(b) **Slit seed coat and soaking in water** The germination of air-dry seeds, which were harvested on the 19 th of May in 1967, was tested on the 22nd of May. Before the test, following pretreatments were carried out : (1) A part of seed coat was slit with a razor blade ; (2) Seeds were soaked in running water during 17 hours after the seed coat was slit ; and (3) Intact seeds as a control. The results are shown in Table 3. Germination was accelerated by slitting of seed coat and further by soaking seeds.

Table 3. Effect of slitting of seed coats and soaking in running water on the breaking of dormancy in *B. japonica* seeds.

Treatment	Number of seeds	Percentage germination (%)
Seed coat slit, not soaked	50	54
Seed coat slit, soaked	50	64
Intact, not soaked	50	4

(c) **Bare embryos and soaking in water** Germination tests were made immediately after harvesting on the 19 th of May in 1967. Following pretreatments were carried out ; (1) Embryos were made bare by the entire removal of seed coats ; (2) Bare embryos were soaked in running water during 17 hours ; (3) Intact seeds were treated as (2); and (4) Normal untreated seeds. Results are shown in Table 4. Dormancy could be broken to some extent

Table 4. Effect of the removal of seed coats and soaking in running water on the breaking of dormancy in *B. japonica* seeds.

Treatment	Number of seeds	Percentage germination (%)
Seed coat removed, soaked	50	100
Seed coat removed, not soaked	50	18
Intact, soaked	50	0
Intact, not soaked	50	0

by only removing seed coats, but the bare embryos showed 100% germination when they were soaked in water. The soaking of intact seeds had no effect on breaking dormancy.

6. Inhibiting effect of seed debris on germination

In order to see whether dormancy is due to germination-inhibiting substance or not, following experiment was carried out. One gram of dormant seeds harvested on the 19th of May in 1967 were ground into powder with 8 cc of distilled water in a mortar. The debris of the seeds were spread on blotting paper in a petri dish, and then non-dormant seeds produced in 1966 were placed on the debris. As a control, the same non-dormant seeds were placed on the debris of non-dormant air-dry seeds which were harvested on the 20th of May in 1966. A germination test was begun on the 25th of May in 1967, but no seeds germinated in both

Table 5. Germination of seeds on the debris of dormant and non-dormant seeds.

Media	Number of seeds	Number of germinated seeds						Percentage germination (%)
		May 26th-30th	May 31st	June 1st-10th	June 11th-20th	June 21st-30th	Total	
The debris of non-dormant seeds produced in 1966	50	0	Washed	22	16	1	39	78
The debris of dormant seeds produced in 1967	50	0	Washed	1	6	0	7	14

treatments. On the 30st of May, the ungerminated seeds were washed and transferred to new petri dishes containing no debris. The results are shown in Table 5. The non-dormant seeds showed low germination when placed on the debris of dormant seeds as compared with the seeds on the debris of non-dormant seeds.

Discussion

After harvest, *Brassica japonica* seeds showed prolonged dormancy for as long as more than three years when stored in the desiccator, while their dormancy disappeared in about two months when stored in the room (Fig. 1). Such a phenomenon has not been reported at least in other *Cruciferous* vegetables. On the contrary, there is a report[6] that the dormancy of *Cruciferous* seeds may be overcome in a short time if they are abruptly dried immediately after harvesting. TOKUMASU (unpublished) also observed that the removal of dormancy was hastened by desiccation in *Brassica napus* seeds. So it seems that the prolongation of dormancy by desiccation is a peculiar phenomenon to *Brassica japonica*. On the other hand, there are some kinds of plants in which the dormancy of their seeds may be overcome by means of so-called "moist low-temperature stratification." For example, the seeds of *Aralia*[5] and *Portulaca*[10] exhibit dormancy during a long period after harvest if they are kept in a dry condition at room temperature, but they become readily germinative if stored in cold wet sand. In such cases where moisture and low temperature are necessary for after-ripening of seed, dormancy is a usual state of seed if untreated and left intact after harvesting. In *Brassica japonica* seeds, however, the dormant state is only temporary when they are left in ordinary air-dry condition. Thus there seems to be a difference in the effect of dryness upon dormancy between both types of plants.

During the period of prolonged dormancy, desiccated seeds showed a continued fluctuation in percentage germination, ranging from 5% on one occasion to 80% on another occasion (Figs. 1 and 2). There are certain plants which exhibit a remarkably uniform periodicity in germination. For instance, seeds of *Amaranthus retroflexus*[1] held moist in a 20°C room for 78 months after harvest had two large germination pulses at 10th and 20th month of storage. The periodicity under such controlled conditions was apparently independent of external conditions. In the present case of *B. japonica*, however, germination tests were made at room temperature changeable with season and weather. So it is questionable whether or not *B. japonica* seeds have periodicity in germination as endogenous trend, but the fluctuation in the germination of desiccated seeds may be partially ascribed to external conditions, especially room temperature. Fig. 3 clearly shows that the percentage germination of desiccated seeds is more influenced by temperature than that of air-dry seeds.

KAWAHARA and WATANABE[6] stated that the depth of dormancy of *Cruciferous* seeds varied according to the stages of their development: the seeds which were harvested in immature state showed less dormancy; the dormancy became deeper as the maturity of seeds proceeded; and over-mature seeds were again exhibited to be less dormant. Such a tendency was also recognized in leaf mustards by SUGIYAMA and HORI[11]. TAKIGUTI[12] tested the germination of rape (*B. Napella*) seeds harvested every 10 days from 30th (milk stage) to 70th day (complete ripening stage) after flowering. And he found that the germinating capacity of the seeds decreased markedly at about 50 days after flowering (fully developed embryo stage) and the decreased germination continued to later stages. These investigations suggested the change of dormancy from immature to mature states of *Brassica* seeds. In the present case of *B. japonica*, there was no difference in the depth of dormancy among air-dry seeds with different maturities. KIDD and WEST[8] found that excised embryos in *B. alba* germinated freely in all stages of development from immature to fully mature, though the testa of immature seeds prevented germination and the seed coat became less effective in preventing germination as the seeds ripened. In *B. japonica*, dormancy is not affected by the degree of maturity of seeds whether the dormacy may be caused by seed coats or by embryos themselves. However, it is interesting that desiccation effect of seeds on dormancy varies with the different degree of maturity. The earlier the stage of development of seeds is, the more deeply their dormancy is maintained when the seeds are desiccated.

It is often seen in many kinds of vegetables that the germination of non-dormant seeds is inhibited by the application of high concentrations of carbon dioxide in the atmosphere[3,7]. When the inhibiting carbon dioxide is removed, the seeds usually germinate in the normal way. In *Brassica alba* seeds, however, the dormancy is induced by carbon dioxide and it continues after the removal of the inhibiting gas[7]. This induced dormant condition following as an "after-effect" of carbon dioxide inhibition is termed "secondary dormancy[7]." Induction of secondary dormancy by the treatment of oxygen dificiency as well as high pressures of carbon dioxide was also reported[3] in the seeds of radish and *Brassica* vegetables other than *B. alba*. In *B. japonica* seeds, the breaking of their dormancy is inhibited by desiccation. When desiccated dormant seeds are transferred to air-dry condition, their dormancy disappears in about four weeks (Table 1). Once their dormancy has been broken, they never return to the quondam dormant condition even if they are again brought into the desiccator. That is to say, desiccation cannot induce "secondary dormancy." It may be considered that desiccation does not cause dormancy, but only maintain it, whether it may be primary or secondary. The effect of desiccation upon dormancy in *B. japonica* seeds, therefore, is essentially different from that of carbon dioxide in *B. alba* and other *Brassica* seeds. In addition, KIDD and WEST[7] observed that secondarily dormant seeds of *B. alba* which lay on damp sand during 12 months continued to germinate in a sporadic manner. The same slow sporadic germination is seen in the prolonged dormant seeds of *B. japonica* as shown in Table 1, where a long period of 7 to 9 months was required for the complete germination of whole seeds. On this point, dormant state is similar in both *Brassica* seeds.

Some workers considered that the cause of dormancy lies in seed coats. TAKIGUTI[12] stated that new seed coats prevent carbon dioxide and other injurious substances from running off from rape seeds. HORI and SUGIYAMA[3] came to the same conclusion in leaf mustard seeds. While, COX *et al.*[2] described that the delay in germination of cabbage seeds is not due to the restriction of gas exchange but due to some inhibitor in the seed coats. HORI and SUGIYAMA[3] also indicated the possibility of the presence of a germination inhibitor. The rate of germination in dormant seeds of *B. japonica* was increased by the removal or slitting of seed coats (Tables 3 and 4). These results suggest that seed coats are strongly related to dormancy. However, it cannot be decided whether seed coats prevent gas exchange or not. When bare embryos were washed by running water, 100% germination took place (Table 4). Soaking is also effective for germination of seeds with slit seed coats (Table 3) but not for that of intact seeds (Table 4). Such an increase in germination can be explained if it is assumed that some inhibitor in seed coats or in embryos may be washed away and released into water. A germination inhibitor or inhibitors must be contained not only in seed coats, as indicated by other workers, but also in embryos themselves. And seed coats, if flawless, perhaps prevent leaching of such inhibitors as shown in Table 4. The presence of germination inhibitors is demonstrated by the difference in germination of non-dormant seeds placed on the debris of new and old seeds (Table 5). In Table 5, germination was inhibited in both plots at first perhaps owing to excessive inhibiting substances oozing from the debris, but, after washing, the seeds on the debris of old (non-dormant) seeds showed a high germination as compared with those on the new (dormant) seeds. It can be said that dormant seeds had more inhibitors than non-dormant ones.

Dormancy disappears in one and a half months after harvest if seeds are stored in an air-dry condition, and thus almost all seeds become capable of germination at the end of June (Figs. 1 and 2). In practice the percentage of germination is not problematic at the time when seeds are sown in late summer or in autumn, so long as the conventional method of seed storage is used. But if freshly-harvested seeds are stored in the desiccator with the aim of increasing the life span of seeds, they will fail to germinate owing to their dormancy even at the sowing season. Special attention must be paid to this point during the practical procedure of seed germination in *Brassica japonica*. Among the tried methods for increasing the germination of dormant seeds, soaking is ineffective if it is not accompanied with the removal of seed coats. Removing and slitting of seed coats are troublesome and unpractical. Low temperature treatment seems to be most useful, but more detailed investigations are necessary to establish the best way of breaking the dormancy of seeds in *Brassica japonica*.

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乾燥貯蔵によるミズナ種子の休眠持続

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摘　要

ミズナ *Brassica japonica* SIEB. の種子は通常収穫後約2か月の休眠期間があるが，これをデシケーター中に乾燥貯蔵するとその休眠は延長持続され3か年以上に及ぶ。乾燥による休眠は完全なものではなく，その間の発芽率の推移に変動がある。乾燥によつて成熟種子のみならず未熟種子も休眠が延長されるが，後者は前者よりも休眠が深いようである。デシケーター中の休眠種子を室内に取り出すと休眠は約4週間で完全に覚醒（せい）するが，一旦休眠の覚醒（せい）した種子を再びデシケーターに入れても休眠に入ることはない。発芽に際して，乾燥種子は室内保存種子よりも温度に敏感である。休眠覚醒（せい）の手段としては低温処理，傷皮および剝皮処理，流水浸漬処理などがある程度有効である。