コウヤマキ種子の休眠

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論　文

Dormancy of *Sciadopitys verticillata* Seed*

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要　旨：コウヤマキの種子に種々の前処理を施したが，休眠が深くて容易に打ち破ることができなかった。硝酸銀処理を試みたところ，0.02～0.1% 溶液に1昼夜浸しておくことによって発芽に導くことができた。

硝酸銀処理と種子に内在する生長抑制物質との間に相互関係があると考え，メタノールさらに酢酸エチル可溶物質をイソプロパノール・アンモニアによるペーパークロマトグラフィーとアベナ伸長テストを並用して検定したところ，Rf 0.8～0.9 を中心として抑制帯が出現した。硝酸銀処理した種子と水に浸しておいた種子との比較では，抑制帯の活性に顕著な差がなかった。

Summary: The seeds of *Sciadopitys verticillata* SIEB. et ZUCC. are characterized as the deep dormant seeds. Although some pretreatments were tried to promote germination, they were not useful at all. Among the pretreatments tested, the pretreatment with 0.02–0.1% silver nitrate for 24 hours was partially successful for germination of seeds.

The methanol- and ethylacetate-soluble inhibitors were assayed by *Avena* coleoptile straight growth test. The inhibitor zone was found in the histogram of Rf values of 0.8 to 0.9 with isopropanol : ammonia solvent. In the comparison of silver nitrate-treated seeds with water immersed seeds, no significant difference in inhibitor zones was found.

Introduction

It is well known that seeds of Japanese umbrella pine (*Sciadopitys verticillata*) are deep in a dormant state and that their dormancy is not so easily broken by the stratification, i.e. pre-chilling (BARTON, 1930). When the seeds were sown without pre-treatment in the nursery bed in spring, only a part of the seeds germinated in late summer, and most of them were likely to remain dormant. It is also known that the seed coats contain a large amount of phenolic compounds (HATANO and NAKAMURA, 1967).

The present study was undertaken to examine the effects of pre-treatments on breaking of dormancy and also to elucidate the mechanism of deep dormancy.

Materials and Methods

Seed materials: The seeds of *Sciadopitys verticillata* SIEB. et ZUCC., used mainly in the experiments, had been collected in the province of Osaka Regional Forestry Office in 1964, and stocked in the Kameyama Seed Repository. Since receipt of the seeds in March 1966, they were stored in a glass bottle at 5℃ until used for experiments. The percentage of filled seeds per sample seeds was 65%. The description of other sources is made in Results according to the necessity.

Germination test: The germination test was carried out from May, 1968 to April, 1970. Unless otherwise stated, the seeds were germinated on filter paper with underlaid moistened vermiculite in PETRI dishes, which were previously sterilized in a KOCH's steamer or an autoclave. The seeds were germinated in the dark or in the continuous light at 500 to 1,000 lux with daylight fluorescent lamps. Germination temperature was maintained at 25℃ both in the light and the dark. The seeds for dark plots were counted under green safe light (10 lux). For pre-chilling treatment, PETRI dishes containing moistened seeds were covered with black clothes and laid at 2～5℃ for a certain period. Each plot consisted of 50 seeds, and two plots were used for one treatment. At the end of the germination test, the ungerminated seeds were cut with razor blade, and the germination percentage was obtained with germinated seeds as against filled seeds.

Various pre-treatments tested for germination (germination period: 4 weeks) were as follows:

1. Pre-chilling for 2 months
2. Cutting of both tips of the outer seed coat
3. Pre-chilling of the above described cut-off seed for 3 months

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4. Immersion in warm water at 45° for 1 to 5 days
5. Compound stratification (25℃– one week and 5℃– one week, repeating 1–7 times
6. Immersion in 1% H_2O_2 at 25℃ for 1 to 5 days
7. Immersion of the intact seed and the cut-off seed in 50 ppm gibberellin* solution at 25℃ for 1 to 4 days
8. Immersion in 100, 200, 500 and 1,000 ppm solutions of gibberellin and potassium indole-3-acetate at room temperature for 24 hours
9. Immersion in 0.1% silver nitrate for 1 to 15 minutes and then cleaning with water for 10 minutes (HATANO, 1967)
10. Immersion in 0.01% silver nitrate solution for 1 to 4 weeks.

Pre-treatments above mentioned did not show any success in seed germination.

Assay for inhibitors in the seed: The chromatographic separation of growth substances and the *Avena* coleoptile straight growth test were modified from those established by BENNET-CLARK and KEFFORD (1953).

Two grams of the dormant seeds (80–90 individuals) were mashed in the mortar, cleansed with petroleum ether three times, and homogenized in 30m*l* of cold methanol, and then the supernatant was decanted. The residue was homogenized once more in 20 m*l* cold methanol. The supernatant was added to the homogenate and laid at 5℃ overnight. The homogenate was filtered off, and after 50 m*l* of methanol was newly added to the residue, the suspension was laid at 5℃ for 24 hours. The supernatant was filtered. The combined filtrate was concentrated to dryness under reduced pressure with stream of small volume N_2 gas below 35℃.

Inasmuch as the methanol extract was not well developed on paper chromatography, the extract was dissolved with 20 m*l* of water and filtered, and the filtrate was shaken with 50 m*l* of ethylacetate two times. The combined ethylacetate partition was added with anhydrous sodium sulfate and laid overnight. After filtration and concentration, a small amount of ethylacetate was added to the dried extract and loaded on a strip of chromatographic paper (Toyo Roshi No. 51 A) of 12 cm in width.

The chromatograms were developed with isopropanol : 28% aqua ammonia : water (10 : 1 : 1) and n-butanol : 28% ammonia : water (8 : 1 : 1). The solvents moved around 30cm long at room temperature to the descending direction. The filter paper was cut into two pieces of 10 cm and 2 cm wide. A piece of 10 cm wide paper was used for the bioassay and 2 cm wide paper for the colour tests (HATANO, 1971).

The straight growth bioassay with coleoptile of oat (*Avena sativa* L., Victory I) (NITSCH and NITSCH, 1956) was modified in the present experiments. The seeds, whose husks were removed previously, were imbibed in water for 2 hours and then, after washing, they were sown on filter paper on 45° angle slope of wet sand in a box under room light. Following the method by WIEGAND and SCHRANK (1959), the seeds were treated with red light (150～200 lux) for 48 hours and then in darkness for 24 hours at 25℃. After 74 hours, the 20～25 mm coleoptiles were selected and were cut into 4 mm pieces between 4 and 8 mm from the tips, accompanied with primary leaves. The sections were immersed with de-ionized water for 3 hours.

Chromatogram paper of 10 cm in width was cut into 10 uniform sections along the running direction, and each section was further cut finely. They were placed in small glass tubes together with 10 coleoptile sections and 2m*l* of de-ionized water and left for 24 hours at 25℃ in the dark.

The manipulation to cut sections was carried out under green lamp (10 lux). The final coleoptile length was measured by a scale lupe (x 10). The coleoptile growth percent was shown by the rate of ten value average of the final length minus the initial lenght (4mm) to the control with water, without filter paper.

Results

Although one or two seeds germinated after the following pre-treatment, their radicles did not bend geotropically: both tips of the outer seed coat were cut off, or additionally both backs of the outer seed coat were slitted, and then the seeds were immersed in 0.1% silver nitrate solution for 1 to 4 hours and cleansed with running water for one hour and with aseptic water for 5 minutes. Therefore, the pre-treatment of silver nitrate was modified and used for the intact seed. Namely, immersion time in the solutions was changed into 24 hours at room temperature and cleansed with running water for 30 minutes and aseptic water for 5 minutes. The results of germination, combined with other treatments, were shown in Table 1 and 2.

From the result of Table 1, it is clear that silver nitrate treatments of an appropriate concentration and period were effective for germination of the seeds and that gibberellin did not show any additional effect on silver nitrate in the combined use. And, it is noteworthy that the seeds did not germinate in the light. Table 2 shows that the pre-chilling within 3 months reduced the effectiveness of silver nitrate pre-treatment.

* The gibberellin used in this study was supplied from the 'Nippon Gibberellin Kenkyu-kai'

Table 1. Germination of seeds treated with silver nitrate and gibberellin

Germination period (Weeks)	Germination percentage																	
	$AgNO_3$ (%)						Gib. 100ppm+$AgNO_3$ (%)						$AgNO_3$ 0.02%+Gib. (ppm)						Not treated	
	0.1		0.05		0.02		0.1		0.05		0.02		1,000		500		200		L	D
	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D		
4	0	2	0+2*	1	0	0	0	1+1*	0	1	0	0	0	0	0	0	0	0	0	0
8	0	16+2*	0+2*	21	0	11	0	10	0	10+1*	0	6	0	1	0	8	0	0	0	0
10	0	26+3*	0+2*	37	0	57	0	23	0	19+1*	0	41	0	16	0	24	0	19	0	2

Gib.: gibberellin
L: continuous light; D: darkness; *: protruding root did not yet bend geotropically

Table 2. Germination of seeds treated with silver nitrate and then pre-chilled

Germination period (Weeks)	Germination percentage									
	Treated with $AgNO_3$ (0.02%), then pre-chilled								Not treated, pre-chilled	
	Weeks									
	0*		4		8		12		12	
	L	D	L	D	L	D	L	D	D	
4	0	0	0	0	0	0	0	0	0	
8	0	11	0	0	0	0	0	0	0	
10	0	57	0	1	0	0	0	1	1	

*: quoted from Table 1.

Table 3. Germination of seeds ever-moistened with silver nitrate and gibberellin

Germination period (Weeks)	Germination percentage						
	$AgNO_3$ (%)			Gibberellin (ppm)			Not treated
	0.01	0.005	0.002	100	50	20	
4	0	0	0	0	0	0	0
8	0	0	0	0	2	0	0
10	0	2*	11	0	6	1	3

Germination bed of de-fatted cotton was moistened with the solutions of silver nitrate or gibberellin
The test was carried out in the dark
*: root tip was damaged by the sorrounding silver nitrate solution

Table 4. Germination of seeds of other origins treated with silver nitrate

Germination period (Weeks)	Germination percentage											
	I			II			III			IV		
	$AgNO_3$ (%)											
	0.05	0.02	0	0.05	0.02	0	0.05	0.02	0	0.05	0.02	0
4	0	0	0	0	0	0	0	0	0	0	0	0
8	11	4	0	12	14	0	4	0	0	16	14	0
10	34	20	4	59	65	11	32	36	5	50	53	6

I: same origin as in Table 1~3
II: collected in Nagano province (Otaki forestry office) in 1965, and received in Feb. 1968
III: collected in Nagano province (Magome forestry office) in 1965, and received in Jan. 1968
IV: collected in Gifu province (a private forest) in 1969, and received in Nov. 1969
The test was carried out in the dark

Table 3 shows the effect of silver nitrate and gibberellin on seed germination by ever-moistening with these chemicals, and Table 4 shows the effect of silver nitrate pre-treatment for seeds of other sources. Although a part of the result in Table 4 was somewhat different from that in Table 1, it is shown that the pre-treatment of 0.02% and 0.05% silver nitrate for 24 hours is effective to break the dormancy of *Sciadopitys verticillata* seed.

Assay for inhibitors in the seeds: The following results of bioassay for inhibitors were obtained in relation to the breaking of dormancy with silver nitrate treatment.

From the result in Fig. 1, it is shown that the seed contains a growth inhibitor, whose Rf value corresponds to that of a phenolic compound coloured with bis-diazotized benzidine (HATANO, 1971).

Fig. 2 shows a comparison of the extract from the seed treated with silver nitrate with that of seed immersed in water. The difference between levels of inhibitor zone was not distinguished.

Discussion

The deep dormancy of tree seeds is an interesting problem in respect to the ecological adaptation of forest trees with propagation in natural stands. Recently, NIKOLAEVA (1969) has emphasized the significance of stratification of long period for the breaking of such dormancy in many woody species.

In the present study, the germination of *Sciadopitys verticillata* seed was not stimulated by the pre-chilling within 3 months. Furthermore, although many trials to seed coats of *Sciadopitys verticillata* were tried for breaking of dormancy, no effective treatments were found.

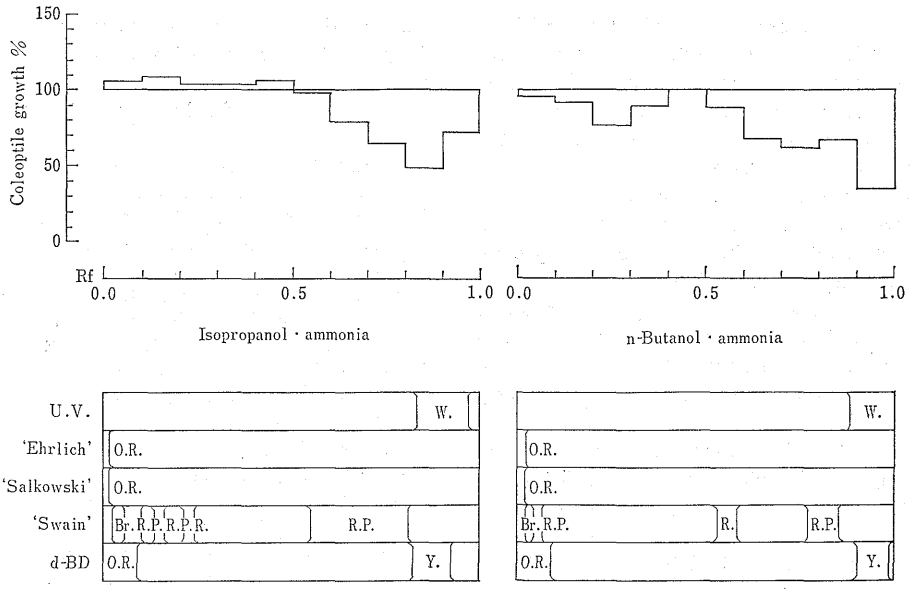

Fig. 1. Activities of bioassay on chromatograms of ethylacetate partition and colour tests with phenolic compounds
Horizontal line: control; abscissa: Rf in isopropanol • ammonia (left) and n-butanol • ammonia (right)
Colour: W.=white, O.R.=orange red, Br.=brown, R.=red, R.P.=reddish purple, Y.=yellow

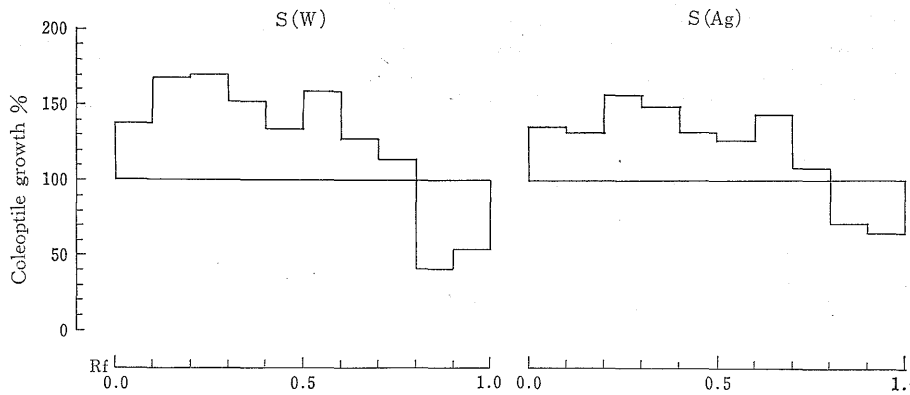

Fig. 2. Comparison of bioassay activities on chromatograms of ethylacetate partitions between seeds treated with silver nitrate and water
S(W)—intact seeds imbibed in water at 25℃ in the dark for 24 hrs.
S(Ag)—intact seeds imbibed in 0.1% $AgNO_3$ similarly for 24 hrs., then rinsed with running water 30 min. and de-ionized water 5 min

Pre-treatment with 0.1% silver nitrate for 24 hours stimulated the dark germination of *Sciadopitys verticillata* seed, that is, for treated seeds, 2% in 4 weeks, 16% in 8 weeks, and 26% in 10 weeks, but for untreated seeds, 0% in 4 and 8 weeks, and 2% in 10 weeks. Similar pre-treatment for 5 minutes was effective for germination of the *Pinus densiflora* seed, as shown in the previous paper (HATANO, 1967).

NIKOLAEVA did not so much refer to growth inhibitors in tree seeds. There may be a profound relationship between the deep dormancy of seed and the endogenous germination inhibitors (WAREING,

1969). In fact, the seed of *Sciadopitys verticillata* contains growth inhibitors detected by *Avena* growth assay, similar to those which were found in the seed of *Pinus densiflora* (HATANO, 1971). Although the values of bioassay could not be compared at the same time between seeds of *Pinus densiflora* and *Sciadopitys verticillata*, it was estimated roughly from weight of used seed samples that the *S. verticillata* seed contains about five times as much of growth inhibitors as does the *Pinus densiflora* seed.

Both conifer seeds were stimulated with silver nitrate and they have the same inhibitors to *Avena* coleoptile growth, but the tests did not corroborate the positive difference in the inhibitory activity between treated and untreated seeds.

It may be that the inhibitor zone in chromatogram contains complex of inhibitors, one of which inhibits the growth of *Avena* coleoptile, but the inhibition does not diminish with silver nitrate, and that the other one, a germination inhibitor, has a relation to silver nitrate.

The present study demonstrates that the treated seeds germinated only in the dark. Silver nitrate remaining in the seeds seems to become invalid as a mitigative for the inhibitor in the light. Similarly, the negative effect of pre-chilling on the silver nitrate treated seeds might be elucidated on the inactivation of silver nitrate for the inhibitor in seeds by low temperature kept for a long period.

Understanding of the nature of the inhibitors in the seeds and functions of silver nitrate for breaking dormancy depend on further studies.

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