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Genecological Studies on Japanese Lawn Grass (*Zoysia japonica* Steud.) with Special Reference to the Seed Propagation Characteristics

(4) Germination of Seeds from Representative Clones*

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SUMMARY

Some laboratory experiments were conducted to examine the germination response of *Zoysia japonica* in relation to the seed dormancy. The seed used were derived from the representative clones categorized previously into the three population groups taking the germinability of the seeds into account, that is easy(A), intermediate(B), and difficult(C). These seeds were given the cool-dry storage for 30-36 months after harvest.

In Experiment 1, the germination response to chilling temperature was re-examined. The result indicated that 10°C, as well as the usual chilling temperature of 5°C, obviously affects on germination acceleration, and that 15°C was also effective to some extent for this grass species. Based on the result, 10°C-20 days was adopted for a prechill treatment in the following experiments.

In Experiment 2, the germination response to temperatures ranging from 15°C to 30°C with 5°C increments was compared. Seeds tested underwent a combined set of physico-chemical pretreatment for breaking the primary dormancy. The result showed that the sensitivity of seeds to temperature-difference increases in the order of the seeds from Group C, B, and A. For instance, the Group A seeds germinated fairly well even at 15°C, whereas the Group C seeds substantially failed to germinate at this temperature. These results suggested that the environmental control of germination, the so-called "environmental dormancy" or "thermo-inhibition", became more rigid as coming down the germination category.

In Experiment 3, the effects of incubation in unfavorable conditions on the subsequent germination process were examined. The seeds used in this experiment were given a treatment by NaOH alone. In Group A seeds, the primary dormancy was broken by this treatment. Although the incubation under unfavorable conditions caused a significant reduction in germination of this group, a further germination occurred immediately after moving ungerminated seeds into favorable conditions. In contrast with this, the single treatment of NaOH broke the primary dormancy of Group C seeds only partially. Unfavorable incubation conditions resulted in nearly complete inhibition of germination. Recovery from this inhibition by subsequent incubation at the favorable conditions, however, was

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rapid as in Group A. These results suggested that the Group C seeds required much more intensive treatments for complete loss of their primary dormancy, and that the subsequent incubation under favorable conditions promote the breaking persistent primary dormancy interacting with the foregoing incubation. On the other hand, secondary dormancy otherwise expected did not occur in either experiment 2 or 3.

Based on the results of the present study, the authors tentatively concluded that the timing of germination of easy germinable seeds depends relatively upon regulation by a short term primary dormancy and by a flexible environmental control. Whereas that of the seeds that germinate only with difficulty depends basically upon the regulation by a persistent primary dormancy with a rigid environmental control. Seeds with intermediate germinability might be regulated variously by the both mechanisms mentioned above. An agronomical and ecological implications of the present study were briefly discussed.

INTRODUCTION

The gemination characteristics of *Zoysia japonica* seeds, in relation to the seed propagation of this species, are one of the important and troublesome problems from the agronomical and ecological point of view. In a series of the genecological approach to this problem, the present authors gave evidence to suggest that a significant difference both between and within local populations in terms of the "germination value" of their seeds. The local populations tested were classified into three groups, Group A, B, and C, which were categorized by easy, intermediate, and difficult germinability of their seeds. In general, they were also characterized by their southern, central, and northern origin, respectively^{1,2)}.

In the present study, the authors conducted some laboratory experiments to clarify in more detail the germination response in relation to the dormancy of seeds derived from the representative clones belonging to different germination category.

MATERIALS and METHODS

In the previous study, 39 local populations containing 263 clones were classified into three groups according to the germination value(GV) of their seeds²⁾. Group A, B, and C were composed of 53 clones of 8 populations, 138 clones of 19 populations, and 72 clones of 12 populations, respectively. The seeds from these groups were characterized by their easy, intermediate, and difficult germinability²⁾. Materials used in the present study were the seeds produced by some representative clones of the three groups.

Seeds were produced by open-pollination under uniform field conditions and harvested at maturity in early August 1987, and stored under cool(ca. 5°C) -dry conditions until they are used. The experiments were carried out from March to October, 1990. Details for the particular experiment are as follows.

Experiment 1.

The seeds produced from a clone No. 1-6 of Group C with GV of 0.23 were used. They were about 31 month-old. Moist seeds were placed under 5, 10, and 15°C temperature conditions for 10, 20, and 30 days each. Chilled seeds were treated by NaOH+(GA₃+KNO₃) in the manner described in the previous paper³⁾. Germination test was carried out under 25°C-light conditions for 7 days using the dry seeds undergoing the same chemical treatment as a control.

Experiment 2.

Seeds from 10 clones of 5 populations in Group A (mean GV = 134.5 ± 45.4), those from 11 clones

of 7 populations in Group B(mean GV = 26.7 ± 3.0), and those from 10 clones of 7 populations in Group C(mean GV = 0.2 ± 0.2), were used. These were about 34 month-old.

A pretreatment combined chilling (10°C-20 days) with chemicals (NaOH + GA₃ • KNO₃) was applied prior to the test. Treated seeds were incubated at 5°C increments over a range extending from 15 to 30°C for germination test. For the control, seeds without any treatment were tested for 7 days under 35°C-light conditions.

Experiment 3.

Seeds from 3 clones of 3 populations in Group A(mean GV = 142.9 ± 29.5), those from 4 clones of 4 populations in Group B(mean GV = 27.4 ± 2.3), and those from 4 clones of 4 populations in Group C(mean GV = 0.2 ± 0.1), were used²⁾. These seeds were under a single NaOH treatment before incubation, and were about 36 month-old.

Two temperature (35°C and 25°C) and light conditions (light and dark) were combined to assess the effect of initial incubation conditions on the subsequent germination process. For the first 10 days, the seeds were incubated under each of the four conditions (Stage I). After all ungerminated seeds were moved into 35°C-light conditions, the germination was observed for 7 days (StageII). Ungerminated seeds were then treated again by NaOH and the germination was tested once more under 35°C-light conditions for an additional 7 days (StageIII).

RESULTS and DISCUSSION

1. Experiment 1.

It is well known that a low temperature around 5°C is universally applicable to accelerate the germination in various kinds of plant species⁴⁾. Accordingly, the authors adopted this temperature for chilling *Zoysia* seeds throughout their previous works^{3,5)}. As to *Zoysia* seeds, on the other hand, Yeam *et al.*⁶⁾ have pointed out in their dormancy study of Korean lawn grass (*Zoysia japonica* Steud.) that 15°C rather than the 5-10°C was more effective in this species. To confirm this point, the present authors conducted an experiment using their own material. The results obtained were given in Table 1.

Table 1. Percentage germination of seeds pretreated by different degrees of moist chilling (parental clone used : No. 1-6).

Chill. temp.	5 °C			10°C			15°C			Cont.
Duration(days)	10	20	30	10	20	30	10	20	30	0
Mean	23.7	59.7	83.7	27.3	56.0	84.0	18.3	25.3	33.0	1.3
± S.D.	1.5	8.1	3.2	14.8	12.5	4.4	3.5	4.5	6.2	0.6

Figures indicate the per cent germination under 25°C, light conditions for 7 days.

All seeds were chemically treated by NaOH + (GA₃ + KNO₃) after chilling.

It is clear from the table that 10°C treatment had an obvious effect on germination acceleration in the same way as an usual one (5°C), and that 15°C treatment was also effective to some extent. Thus, it can be said that the *Zoysia* species, as Yeam *et al.* suggested⁶⁾, characteristically respond to a wider range of temperature including 15°C for prechill treatment of seeds.

Based on this result, the authors adopted hereafter 10°C for a chilling temperature instead of 5°C. In view of the fact that the above-mentioned result was obtained from the material the most difficult to germinate, the duration of chilling was reduced from 30 days to 20 days for wider and more efficient application of this treatment.

2. Experiment 2.

The germination response to temperature was examined using the seeds derived from several clones selected from the Group A, B, and C. The results obtained were shown in Table 2. Although the germination at 35°C was not attempted in this experiment, more germination than at 30°C can be reasonably expected in the light of the earlier works^{3,5,7}.

Table 2. Percentage germination of pretreated seeds from representative clones at different temperatures.

Group	Germinability	No. clones used	Temperature regime (°C)				L. S. D. (p : 0.05)	Control (35°C)
			15	20	25	30		
A	Easy	10	49.5(88.9)	86.6(95.3)	96.0(96.7)	97.1(97.4)	12.8	21.7±21.8
B	Intermediate	12	10.6(73.8)	70.2(90.2)	96.1(97.3)	97.3(97.9)	8.4	2.4±2.2
C	Difficult	10	0.7(69.0)	37.7(73.5)	77.2(86.1)	79.2(86.6)	13.2	0.1±0.4

Figures indicate the per cent germination for 7 days, and those in parentheses indicate the final percentage germination for 40 days.

All seeds exclusive of those in control were pretreated by Chill. + NaOH + (GA₃ + KNO₃).

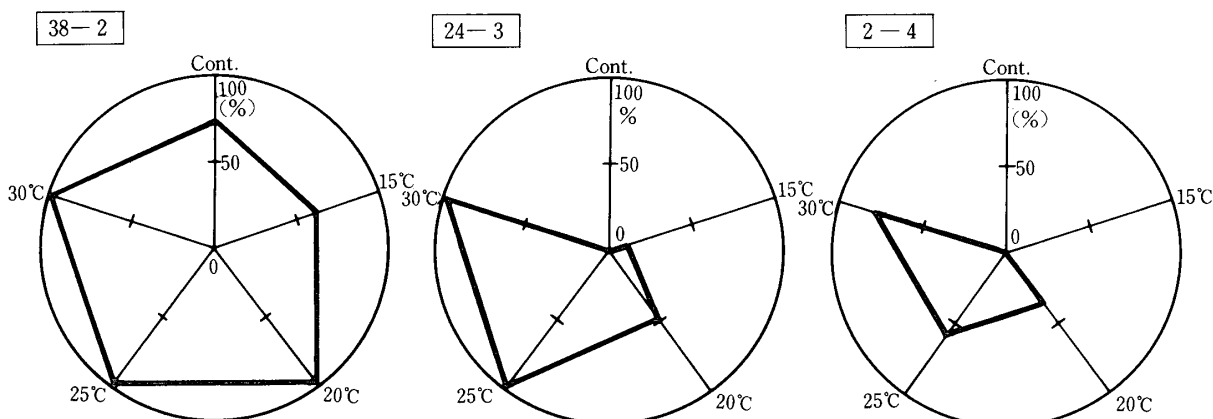


Fig. 1. Germination response to temperature showed by seeds derived from a representative clone belonging to Group A(38-2), B(24-3), and C(2-4).
See also Table 2,

As the final percentage germination at 30°C in Table 2 shows, all seeds used in this experiment had equally high germination potential regardless of the original groups. Nevertheless, the sensitivity of seeds to temperature-differences largely differed from group to group, particularly in lower temperatures.

As Table 2 shows, the Group A seeds germinated over a wide range of temperatures (15-30°C), though the germination at 15°C was significantly less than at higher temperatures. The Group B seeds were also germinable over the same range of temperature as in Group A. In this case, however, germination was strikingly reduced even at 20°C compared with those at higher temperatures.

In contrast to these, the Group C seeds were substantially non-germinable at 15°C. Moreover, the germination at 20°C was much lower than in the other groups. And the germination at higher temperature, too, did not attain the level of the other groups.

Figure 1 illustrates the sensitivity to temperature-differences for the seeds of the representative clones of Group A(clone No. 38-2), B(No.24-3), and C(No.2-4). The parental clones originated from the hilly pastures along the coast of southern Kyushu, the high mountain pastures of central Honshu, and the coastal pastures of southern Hokkaido, respectively¹⁾. From Fig. 1 it is seen that the sensitivity in question characteristically increases with decreasing germinability in the original clone.

These results suggest first that the *Zoysia japonica*, notwithstanding the warm-season type grass, contained seeds which were able to germinate even under the temperature conditions favorable to the germination of cool-season type grasses, when the suitable pretreatment was applied. Thus, a clone like No. 38-2 is considered to be helpful not only to present seed propagation but also to future breeding programs to establish new strains suitable for propagation by seeding of this plant species.

Second, since the primary dormancy of seeds⁸⁾ in this experiment appeared to be well broken by the pretreatment, the reduced germination observed at lower temperatures might be due to environmental influence—the so-called environmental dormancy⁹⁾. This is because no secondary dormancy⁸⁾ might be induced, given the high germination potential at 30°C or more. Environmental or enforced dormancy¹⁰⁾, though not a true dormancy in the strict sense, has no doubt an ecological significance regulating the actual timing of germination as Sawhney *et al.*¹¹⁾ suggested in terms of “thermo-inhibition.” Thus, the Group C seeds drew attention due to their characteristically rigid environmental control of germination owing to their northern origin.

3. Experiment 3.

To observe the germination response from another aspect, NaOH-treated seeds from different groups were tested under various incubation conditions. Unfortunately, the materials tested were largely limited owing to the shortage of available seeds.

During the first 10 days (Stage I), as Table 3 shows, seeds were incubated under each of the four conditions (1-4). Of these, Condition 1 (35°C-light) is known to be optimal for germination of this species, while the others are sub-optimal or somewhat unfavorable^{3,5)}. Immediately after finishing the Stage I, all ungerminated seeds were moved into incubation under the optimal conditions and the germination was observed for 7 days (Stage II). In Group B and C, after finishing the stage II, the ungerminated seeds were treated by NaOH again and the germination was observed for an additional 7 days under the optimal incubation conditions (Stage III).

The results were given in Table 3, and the germination process of seeds from the representative clones in each group were illustrated in Fig. 2.

In Group A, as exemplified by clone No. 33-10 in Fig. 2, almost all seeds germinated by the end of Stage I, when they were incubated under the optimal conditions (35°C-light). On the other hand, the germination was significantly inhibited under the incubation of unfavorable conditions (Conditions 2-4). This inhibition, however, did not continue to the following stage.

On the basis of these data, it seems reasonable to assume that the primary dormancy of the seeds was well broken by the NaOH treatment alone, and that the secondary dormancy⁸⁾ otherwise expected did not induce in this case either, because of further increase of germination at Stage II. In other words, the germination inhibition observed at Stage I was considered also to be largely environmental.

By contrast, a high proportion of seeds remained ungerminated at the end of Stage I even under the optimal conditions in Group C. This means probably that a single treatment of NaOH was able to break the primary dormancy only partially. Therefore, possibility of the secondary dormancy may be set aside. On the other hand, the incubation under unfavorable conditions at Stage I resulted in nearly complete inhibition of germination in Group C seeds. This inhibition, however, also disappeared at Stage II. Further, retreatment of ungerminated seeds with NaOH resulted in an additional germination at Stage II. Some seeds still remained ungerminated at the end of Stage III, but most of them decayed during the continuous incubation.

These results suggest that the seeds of this group require much more intensive pretreatment for complete loss of their primary dormancy than those of Group A. This means that the Group C seeds have by far deeper primary dormancy than those of Group A. The incubation under the optimal

Table 3. Cumulative percentage germination of NaOH treated seeds from representative clones under varied incubation conditions.

Group	Germinability	Incubation stage	Incubation condition				L. S. D. (p : 0.05)
			1	2	3	4	
A	Easy (3)	I	91.9	78.7	59.7	73.2	15.8
		II	98.6	93.9	92.7	96.8	NS
		III	—	—	—	—	—
B	Intermediate (4)	I	46.2	16.7	12.3	19.9	10.7
		II	79.1	72.1	89.0	84.6	NS
		III	83.6	90.7	92.4	93.1	—
C	Difficult (4)	I	13.5	3.4	2.3	6.7	5.3
		II	70.7	62.3	79.1	75.9	NS
		III	77.5	84.3	79.1	78.9	—

Figures indicate the cumulative per cent germination through stages, and those in parentheses indicate the number of parental clones used.

Incubation conditions(1-4) are as follows :

Stage	Incubation condition				Duration (days)
	1	2	3	4	
I	35°C, light	35°C, dark	25°C, light	25°C, dark	10
II	35°C, light				7
III	35°C, light after retreatment of NaOH				7

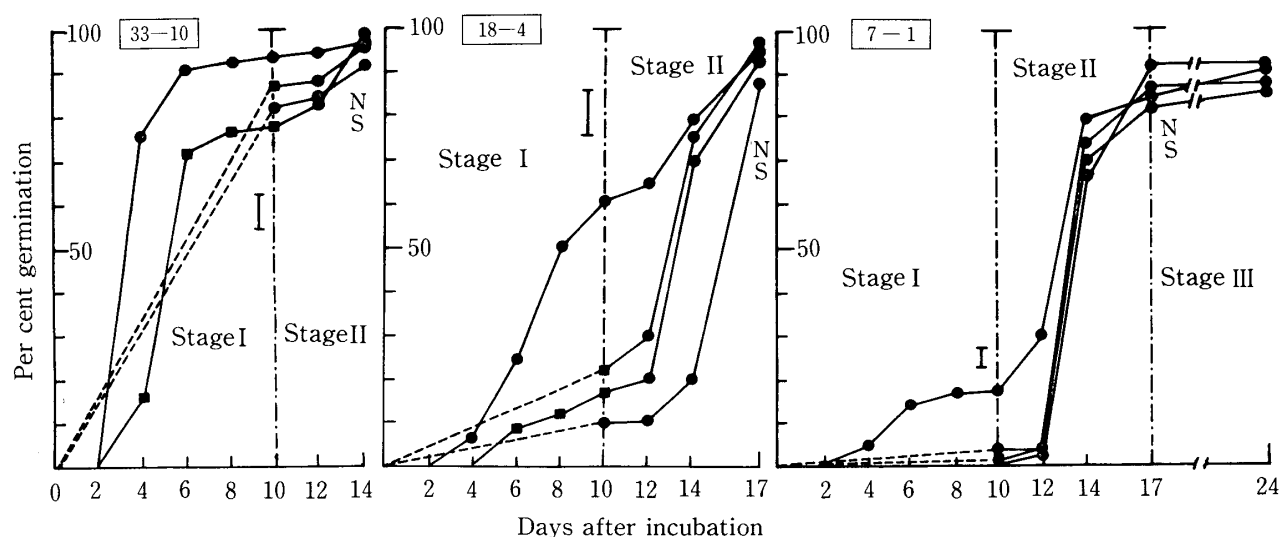


Fig. 2. Germination process showed by seeds derived from a representative clone belonging to Group A(33-10) , B(18-4), and C(7-1) under different incubation conditions.

Incubation conditions are as follows : ●—● 35°C, light ; ●-----● 35°C, dark ; ■—■ 25°C, light ; ■-----■ 25°C, dark. Bars indicate L.S.D. at p : 0.05, and NS indicates non significant. See also Table 3.

conditions at Stage II, interacting with the preceding incubation, seems to promote breaking the persistent primary dormancy in Group C seeds.

Group B seeds, as exemplified by No. 18-4 in Fig.2, are considered to be mid-way between Group A and C in this regard.

Our discussion of Experiment 2 and 3 leads to a tentative conclusion that the timing of germination of easy germinable seeds depends relatively upon regulation by a short term primary dormancy and by a flexible environmental dormancy, whereas that of seeds which germinate only with difficulty depends basically upon the regulation by a persistent primary dormancy accompanied also with a rigid environmental control. Seeds with intermediate germinability may be regulated variously by a moderate degree of both dormancies. Finally, an ecological implication of present study is that the actual germination behavior of seed populations of this species in the field may not be a simple matter. This is a point which must be resolved, along with many others, in the future.

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シバ(*Zoysia japonica* Steud.)の種子繁殖形質に関する種生態学的研究

(4)代表的な栄養系からの種子の発芽反応

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山地開発研究施設

(1991年7月20日受理)

要 約

発芽特性を異にするシバ種子について、休眠性との関連における発芽反応をさらに詳しく検討する目的で2・3の実験を行った。供試種子は、先に著者によって易発芽群(A)、中間群(B)、および難発芽群(C)として分類された地方集団からの幾つかの栄養系に由来し、約30~36ヶ月齢を経ている。

第1実験では冷湿処理の温度を再検討した。その結果、10℃でも従来の5℃と同様の顕著な効果が得られ、15℃でも効果があった。これに基づき、以後の実験には10℃、20日間処理を採用した。

第2の実験では、15~30℃までの5℃きざみの置床温度に対する発芽反応を比較した。供試種子は既報の組合せ処理によって、適温置床(30℃)での一次休眠はいずれもほぼ完全に解消した。しかし、与えられた温度差に対する感受性は発芽難易群ごとに大きく異なり、C→Aの順に環境条件による発芽規則(いわゆる環境休眠、温度抑制)の作用を強く受けることが明らかにされた。

第3実験では、不適当な置床条件がその後の発芽過程に与える影響を検討した。供試種子にはNaOHの単独処理を施した。A群に由来する種子は、この処理によって好適置床下で一次休眠がほぼ完全に解消した。不適当な条件での置床は発芽率を有意に低下させたが、これはその後の好適置床によって向上した。C群由来の種子は、この処理だけではその一部が休眠を解消するに止った。また、不適置床はほぼ完全に発芽を抑制した。しかし、この抑制もその後の好適置床によって急速に解消した。B群由来の種子は、両者の中間的な反応を示した。これらの実験に関する限り、二次休眠誘発の証拠はみられなかった。

これらの結果から、A群由来の種子は発芽のタイミングを相対的に弱い一次休眠とゆるい環境休眠に依存しており、C群のそれは基本的には強固な一次休眠に依存し、これにきびしい環境休眠が加わることで、そしてB群のそれは、中程度の両休眠によって種々に規制されるであろう、という一応の結論が導かれた。農学的ならびに生態学的見地から、これらの結果の含意を考察した。

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