ダイズ種子の冠水抵抗性検定法

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Studies on the Screening Technique for Pre-germination Flooding Tolerance in Soybean

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Abstract : To establish methods for screening of the flooding tolerance of soybean seed, the effects of water conditions and environmental factors on germination were studied both in the laboratory and in the greenhouse. The results indicated that seed damage may be a factor affecting of germintion. We suggested that seeds used for testing should be free from disease and without damaged testa. Significant correlation between data obtained in the laboratory and soil indicated that flooding tolerance of soybean seeds could be evaluated in the laboratory. The procedures for screening soybean seed flooding tolerance were as follows : 1) Seeds were soaked for 4 days at 25°C in the test tube containing 90 m*l* distilled water after sterilization with 70% ethanol for 30 sec. 2) After soaking, seeds were left on dry filter paper for air drying for about 6 hours, 3) Dried seeds were put in the petri dish containing 2 pieces of moist filter paper and moved to 25°C incubators for germination 4) Germination of seeds was determined 4 days after incubation and 5) Flooding tolerance was expressed as a percentage of germination.
Key words : Germination, *Glycine max*, Screen method, Seed flooding tolerance.

ダイズ種子の冠水抵抗性検定法 : 侯福分・曽富生（台湾中興大學農學院）
要　旨 : ダイズ種子の冠水抵抗性検定法を確立するために，種子の浸漬条件ならびに発芽時における環境条件の影響を実験室と温室において検討した．この結果，浸漬によって種子が破る障害が発芽率低下の要因であることが示された．従って，冠水抵抗性検定に供する種子は病害を受けていたり，種子に傷があったりしてはならない．室内実験の結果と土壌に播種した実験の結果は有意な相関関係にあり，ダイズ種子の冠水抵抗性は室内実験で検定し得ることが示された．ダイズ種子の冠水抵抗性検定法は次の通りである．1) 70% エノタールで30秒間消毒した種子を試験管に入れ，蒸留水90 ml を加え25°C で4日間置く．2) 浸漬処理終了後種子を乾いたろ紙上に6時間放置して風乾する．3) 乾燥した種子を湿ったろ紙2枚を敷いたシャーレに置床し，25°C で発芽させる．4) 置床後4日目に発芽率を調査する．5) その発芽率で種子の冠水抵抗性を表示する．
キーワード : *Glycine max*, 検定法，種子の冠水抵抗性，発芽．

Poor emergence in the field under excessive soil moisture conditions is an important factor limiting soybean production in tropical and subtropical areas. Seed damage during germination has been found frequently when soybean is subjected to heavy rainfall after sowing[1]. Failure of seed germination under flooding conditions may have resulted from repid absorption of water by seeds, which disrupts cell membranes[3,13,14,16] and increases leakage of intercellular substances. Since intercellular substances are substrates for pathogen growth, the population of potential pathogens is increased under flooding conditions, thus affecting seed germination.

Planting flooding-tolerant varieties may be the most effective means to overcome the problem of low germination in the presence of excessive water[2]. Genetic variation on seed flooding tolerance has been reported in corn and barley[6,15,18], and high flooding tolerant varieties have been identified in barley and soybean[7,15]. Soybean is recognized as a relatively water-sensitive plant[5] and different responses to moist soil have been reported[1]. To our knowledge, however, there is no study of a massive screening for water tolerance, and methodology for screening flooding tolerance has not been established in soybean. The objective of this study was to establish methodology for screening flooding tolerance of soybean in the laboratory.

Materials and Methods

All soybean varieties used in the experiment were collected from the Taiwan Agricultural Research Institute, and were multiplied over two dry seasons in the field. Seeds were harvested and air-dried until the moisture content was below 11%, and then kept in storage at 5°

C. A standard germination test was used for a preliminary screening. All varieties were found with high germination rate (90% at 7 days after incubation) and subsequently used in the experiments.

Experiment I. Source and rate of water for soaking on seed germination

In this experiment, three varieties : Ottotan, PI088815 and Miyokozima Dzurumame were used. Fifty seeds of each variety with 4 replications were sterilized and pre-germination soaked in the test tube with distilled water, deionized water and ground water. The amounts of water in the test tube were 30, 60 and 90 m*l*, respectively. Four days after soaking, seeds were air-dried on dry filter paper and then put in petridishes containing 2 moist filter papers for germination in a 25°C incubator. Germination rate was recorded 4 days after incubation. Seeds with emerged radicle longer than 1 cm were considered germinated.

Experiment II. Temperature and length of soaking on seed germination

Fifty seeds of Miyokozima Dzurumame were soaked with 4 replications at 5, 10, 15, 20, 25 and 30°C for 2, 4, 6, 8 and 10 days to study the effect of temperature and length of soaking on seed germination. Other 48 varieties were used for further study to identify variation. Fifty seeds of each variety with 4 replications were soaked at 20° and 25°C for 4, 6 and 2, 4 days, respectively. After soaking, the procedures for germination investigation were the same as Exp. I.

Experiment III. Length of air dring after soaking on seed germination

After soaking seeds were air-dried on the dry filter paper for 0, 3, 6 and 9 hours to study effect of length of air dry after soaking on seed germination. Varieties tested and procedures for pre-germination soaking and germination investigation were the same as Exp. I.

Experiment IV. Effect of seed coat cracking

Fifty seeds of three varieties, KS10, PI68586 and PI094173, were used to study the effect of a cracking seed coat on seed germination, seeds with a cracked and intact seed coat were separated. Procedures of germination investigation were the same as Exp. I.

Experiment V. Varietal response to length of soaking

Germinability of 750 varieties were first tested with the standard germination test, and then 730 with high germination (>90%) were chosen for the following test. For each variety, 50 intact seeds (without split or cracked testa) were sterilized with 70% ethanol and then soaked for 2 and 4 days in the tube containing 90 m*l* distilled water at 25°C. Thereafter, soaked seeds were air-dried on filter paper for 6 hours, then put in petri dishes with 2 moist filter papers in the incubator for germination at 25°C. Germinated seeds were counted 4 days after incubation. Frequency of germination for two treatments was compared.

Experiment VI. Seed flood tolerance under soil conditions in greenhouse temperature

Fifty varieties selected randomly from 730 varieties were used. Clay loam soil from a paddy field was crushed and passed through a 0.5×0.5 cm sieve, then poured into plastic basket of 40×30×10 cm.

Thirty seeds of each variety with four replications were sowed 2 cm deep in the basket. After sowing, baskets were moved into an impermeable wooden frame, and water was kept covering the soil during the flooding stage for 2 and 4 days. Percentage of emerging seedling was recorded daily after flooding for 4 days. Seedlings with cotyledon above the soil were considered as emerged.

Results and Discussion

I. Test method

It has been proposed that seed damage under excessive water conditions is due to disruption of cell tissue and lack of oxygen which inhibited respiration, followed by other physiological and metabolic processes[3,4,5,16]. Seed damage as effected by temperature was reported[15]. Therefore, for screening flooding tolerance of soybean collections, factors influencing germination under flooding conditions should be determined in order to establish the screening method.

1. Effect of source and rate of water for soaking

There is an interaction between source of water and variety for germination, as seeds were pre-germination soaked for 4 days (Table 1). A significant difference in germination between ground water and distilled water was found for Miyokozima Dzurumame and PI088815, but not for Ottotan.

Difference in seed damage between distilled and ground water may be related to the presence of water microflora in the latter, which cause seed deterioration[14]. Under flooded conditions, rapid uptake of water may cause the rupture of cell membrane resulting in the leakage of cellular substances from seeds, which may become substrates for potential pathogens. To prevent this pathogen effect, distilled water would be considered as a source for soaking to study the flooding tolerance of soybean seeds.

As shown in Table 2, a relationship between variety and amount of water was found. Germination increased as rate of water increased for PI088815 and Miyokozima Dzurumame but this phenomenon was not found for Ottotan. Different varietal responses to the amount of water may be related to their varied requiremetns of oxygen for germination. In corn, less oxygen was required by flooding tolerant lines[19].

2. Effect of length of drying after soaking

Germination of soaked seeds was affected by the length of air drying after soaking. The germination rate was highest when seeds were air-dried for 6 hours (Table 3). The interaction between length of air drying and variety was also found. Germination was depressed significantly for Miyokozima Dzurumame and PI088815 when seeds were incubated for germination immediately after soaking. However, no significant difference due to length of drying was found for Ottotan. Causal drying, which increased the germinability, may be due to a loss of excessive water from seeds that favors for the repair of ruptured cell membrane[3]. Factors causing the decrease of viability for PI088815 remain to be investigated.

3. Effect of integrity of testa

It has been proposed that testa can serve as a barrier to the diffusion of water and delay water absorption during initiation of imbibition that prevents the repture of the cell membrane[11]. Integrity of seed coat is an important factor that affects the seeds response to environmental conditions[10]. Without an intact testa, the seed imbibes water too

Table 1. Effect of sources of soaking water on the germination of three soybean varieties

Source of water	Miyokozima Dzurumame	Ottotan	PI088815	mean
Distilled water	51.0 a#)	98.5 a#)	73.5 a#)	74.3 ab#)
Deionized water	42.5 a	97.5 a	49.5 b	63.2 bc
Ground water	35.0 b	95.0 a	42.5 b	57.5 c

#) Numbers followed by different letters in a column are significantqy different at 5% level.

Table 2. Effect of amounts of soaking water on the germination of three soybean varieties

Amount of soaking water (m*l*)	Miyokozima Dzurumame	Ottotan	PI088815	mean
30	42.0 b#)	96.0 a#)	48.0 b#)	62.0 b#)
60	50.0 a	94.5 a	48.5 b	64.3 b
90	52.0 a	94.5 a	64.5 a	70.3 a

#) Numbers followed by different letters in a column are significantly different at 5% level.

Table 3. Effect of length of drying after soaking on the germination of three soybean varieties

Length of drying (hr)	Miyokozima Dzurumame	Ottotan	PI088815	mean
0	27.5 b#)	93.5 a#)	5.5 d#)	42.2 c#)
3	41.0 a	99.5 a	15.5 c	52.0 b
6	48.5 a	97.0 a	66.5 a	70.7 a
9	49.0 a	96.0 a	32.0 b	59.0 b

#) Numbers followed by different letters in a column are significantly different at 5% level.

Table 4. Effect of seed coat cracking on the germination of three soybean varieties

Days of soaking	Variety	Intact	Cracked
2	PI68586	99 **	88
	PI087634	97 **	86
	KS10	53 **	2
	Mean	83.0 **	62.6
4	PI68586	62 **	8
	PI087634	60 **	0
	KS10	0	0
	Mean	40.7 *	2.7

**, * Germination of intact and cracked seeds differ significantly at 5% and 1% levels, respectively.

rapidly, creating imbibitional injury[12].

As shown in Table 4, germination was inhibited in cracked seeds regardlesss of the variety. However, the inhibition was greater with 4-day soaking than with 2-day soaking. Results suggested that seeds with damaged testa should be eliminated before testing of flooding tolerance.

4. Effect of temperature and length of soaking

Seed damage due to excessive water may be related to the temperature and length of soaking. At a temperature below 15°C, there is no significant difference in germination as seeds were soaked for 2～6 days. However, seeds were completely damaged with only 4-day soaking at 30°C (Fig. 1). At 20 and 25°C, the damage of seeds increased as the length of soaking increased. High inhibition was found in 4, 6, and 2, 4-day soaking at 20 and 25°C, respectively. After that period, seeds were completely damaged. Differences of germination due to temperature may be related to the rate of water uptake leading to the rupture of cell membrane and, subsequently, leakage of cellular substance from the seed[9,16,17]. Duke et al[4]. reported that the leakage of malate dehydrogenase activity from imbibiting soybean seeds increased as the temperature increased. To identify varietal responses, 48 varieties were tested at 25 and 20°C for 2, 4 and 4, 6 -day soaking, respectively. Results showed that seeds damage was higher at 25°C than at

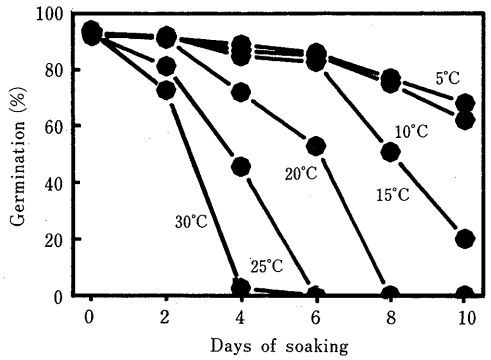

Fig. 1. Effect of temperature and length of soaking on the germination of soybean Miyokozima Dzurumame

20°C. Most of the varieties showed high germination at 20°C for either 4 or 6 days of soaking, and the mean germination was 65.8 and 59.0%, respectively (Fig. 2). Therefore, varietal differences were only poorly distinguished.

Although mean germination between 2-day soaked at 25°C and 4-day soaked at 20°C had no significant difference, the frequency distribution between these two treatments was singnificantly different ($\chi^2=134.77^{**}$). The finding of large varietal variation at 25°C and lower mean germination (25.1%) indicated that 25°C would be better than 20°C for seed pre-germination soaking to evaluate soybean floodng tolerance. It should be noted that temperature (25°C) at which the great variation was found is also the temperature at which most of soybeans have the greatest seedling vigor[4].

II. Varietal response to flooding related to length of soaking

In preliminary experiment, greater variation of germination was obtained when seeds were soaked at 25°C for 2 and 4 days. To compare the frequency distribution of those two treatments, 730 varieties were used.

There was significant difference in relative frequency (Fig. 3). About 15% of varieties showed complete damage by 2 days of soaking, and this was increased to 60% as seeds were soaked for 4 days. Germination of all varieties with high germination (>90%) decreased length of soaking increased, and about 15% of the varieties showed high germination at 2-day of soaking, but this decreased to 4%

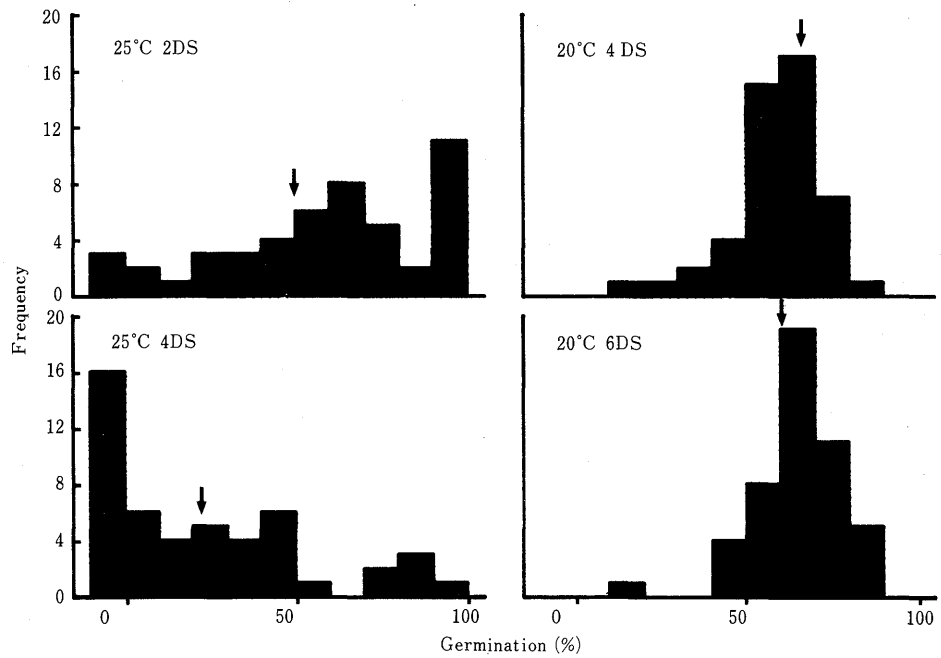

Fig. 2. Varietal variation to flooding tolerance as seeds were soaked at 20 and 25°C for 4, 6 and 2, 4 days, respectively.
(n=48)

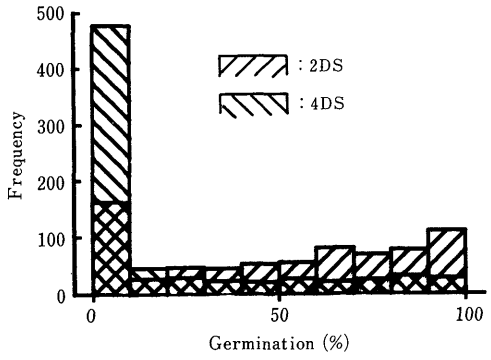

Fig. 3. Comparison of varietial response to flooding tolerance at 25°C for 2 and 4 days of soaking.
(n=730)

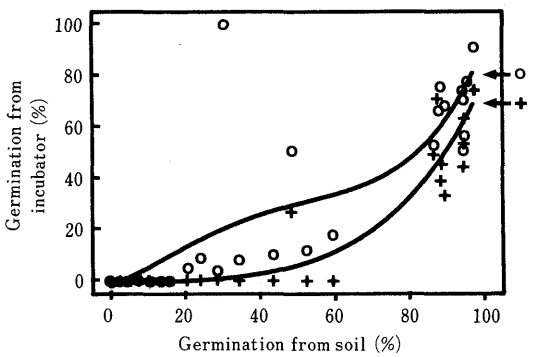

Fig. 4. Relationship between the germination in incubator (25°C) and in soil after 2 days (+), and 4 days (○) flooding.

when seeds were soaked for 4 days. Results indicated that 4-day soaking may serve as a reasonable length of time to evaluate flood tolerance of soybean varieties.

III. Comparison of laboratory test to greenhouse experiment

To examine the relationship between laboratory tests and field data, 50 varieties were tested in soil and flooded for 2 and 4 days. Results obtained from seedling emergence showed that tested varieties could be divided into two groups, i.e. sensitive and tolerant seeds (Fig. 4). Seeds of sensitive varieties were completely damaged with only 2 days of flooding, and those of tolerant varieties produced 50～90% emergence of seedling after 4 days of flooding. Seedling emergence of seeds planted in soil flooded from 2 or 4 days was highly correlated with the germination of seeds with 4-day pre-germination soaking in laboratory

Table 5. Responses of germination parameters to 2-day and 4-day soil flooding

Variety	Germination (%)		Ave. length for germination (days)		Coefficient for germination (%)	
	2-day	4-day	2-day	4-day	2-day	4-day
PI174860	71.1	44.4	5.5	6.5	12.8	6.8
PI165957	77.7	73.3	5.5	6.3	13.9	11.5
PI205913	75.6	38.9	5.1	6.8	14.7	5.6
PI194773	67.8	33.3	5.1	7.2	13.1	4.6
PI208430-1	74.4	53.3	5.1	6.0	14.4	8.7
Miyokozima Dzurumame	51.2	26.7	6.1	6.6	8.3	4.0
PI175184	71.0	66.7	5.3	5.2	12.2	13.5
PI222549	53.3	48.9	5.4	4.8	9.8	10.0
PI222550	63.3	56.7	5.7	4.8	9.8	12.9
PI232989	63.3	51.0	5.4	5.0	9.3	12.4
PI186195	91.0	74.4	5.1	5.1	17.7	14.4
PI181697	66.7	45.6	4.7	7.0	14.1	6.4
Mean	68.9	51.1	5.3	6.0	12.5	9.2

test, but not correlated with data of 2 days of soaking. Nevertheless, mean germination in the laboratory was about 30 percent higher than those in the soil.

Germinating speed, average length of time for germination, and coefficient for germination are presented in Table 5. Length of germination was longer for 4-day flooding than 2-day flooding, indicating that seed emergence was delayed by flooding. Different varietal responses to soil flooding were found, and some tolerant varieties gained 70% more emergence. Similar to the laboratory test, those varieties were highly resistant to a long period of flooding (10 days)[7].

As the days of flooding increased, seed emergence decreased. This result agrees with some reports in corn. In flooded soil, the damage of seed increased as the days of submergence increased. Different responses to flooding may to related to the change of metabolic process. Khosravi and Anderson[8] indicated that ethoanol accumulation was negatively correlated with germinability.

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