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Effects of Acid Scarification, Priming with PEG, NaCl or Sea Water as Osmoticum and Dehydration on Spinach Seed Germination at 30℃

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Summary

Spinach (*Spinacia oleracea* L.) seeds were scarified with an 18 N H_2SO_4 solution, then primed for one week at 10℃ in PEG-6000, NaCl or natural sea water. Germination tests were carried out at 30℃, which is normally inhibitory for spinach seed germination. The germination rate of scarified seeds was higher than the untreated ones. Four successive use of the acid solution did not reduce its scarifying potential; the most suitable soaking time depended on seed size. When seeds were dehydrated and stored for 10 months after scarification and NaCl priming, more than 80% germinated at 30℃. Sea water, which is also effective as a priming agent, could replace either PEG-6000 or NaCl. The priming method developed in this study may be useful especially at high temperatures in summer.

Key Words: acid scarification, NaCl, sea water, seed priming, *Spinacia oleracea*.

Introduction

Spinach seed germination is inhibited at high temperature by the presence of the pericarp that possesses germination inhibitors and reduces permeability to gases (Makino and Miyamoto, 1954; Suganuma and Ohno, 1984). The mechanical removal of the pericarp, or the use of scarifying agents such as sulfuric acid, promotes germination, but above 25℃, seed germination is delayed and restricted even after the removal of the pericarp (Atherton and Farooque, 1983a), which indicates that high temperatures have an adverse effect on the embryo (Suganuma and Ohno, 1984).

Several methods for promoting seed germination under unfavorable conditions are known. One of them is seed priming, which is a controlled hydration treatment that allows pre-germinative metabolic activity to proceed, while preventing radicle emergence (Bradford, 1986). At high temperatures, priming spinach seeds by extended inhibition in osmotic solutions of polyethylene glycol (PEG-6000) markedly improved germination (Atherton and Farooque, 1983b; Nakamura et al., 1982). Osmoconditioning with salt solution instead of PEG-6000 has also been demonstrated to improve the rate and the percentage of germination in various species (Frett et al., 1991). Pansy seed primed with $CaCl_2$ at -0.1 MPa, germinated at a greater rate than did those all treated by other priming regimes, including aerated PEG-8000 solution (Yoon et al., 1997). Treated celery seed can either be sown surface-dried, or after storage in a dry air condition (Heydecker and Gibbins, 1978). A similar treatment has also been shown to increase seed vigor in muskmelon seeds when they are stored for as long as nine years (Oluoch and Welbaum, 1996). For most commercial purpose, primed seeds are dried before seeding. It has been shown that primed and dehydrated seeds maintain a beneficial effect on germination in onion (Dearman et al., 1985), carrot and leek (Dearman et al., 1987), and muskmelon (Oluoch and Welbaum, 1996). However, this procedure is not always applicable to all species. Recently, removal of spinach pericarp is being practiced in Japan, but such treated seeds occasionally give rise to atypical seedlings (Katzman et al., 2001).

Masuda and Konishi (1993) reported an acceleration in the germination rate of spinach seed at 30℃ after acid scarification followed by priming with PEG-6000, but the question as to how many times the acid solution can be re-used was not addressed. Furthermore, the suitability of PEG as a replacement for other salt solutions needs to be established. This study is focused on the efficacy of reusing sulfuric acid as a pre-priming agent for spinach seeds, followed by osmoconditioning with NaCl or sea water versus PEG-6000 and subsequent storage characteristics of the treated seeds.

Materials and Methods

Spinach (*Spinacia oleracea* L.) seeds of 'Jiroumaru', stored in a desiccator at room temperature for one year, were visually divided into large, medium, and small-sized populations; their average weights were 2.7, 1.2,

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and 0.6 g per 100 seeds, respectively.

Effect of recycling sulfuric acid solution as a scarifying agent on seed germination

Two hundred medium-sized seeds were scarified for two hours in a 100 mL 18 N H_2SO_4. After removing the seeds, the same acid solution was used to scarify these fresh lots of 200 medium-sized seeds. Non-scarified seeds (control) were steeped in H_2O for 2 h. Scarified and non-scarified seeds were rinsed in tap water for 30 min, then sown on a layer of moistened filter paper with 3 mL distilled water in 9 cm petri dishes. The petri dishes were incubated in the dark at 30℃ and the filter paper replaced every two days. A seed was considered to have germinated when its radicle elongated over 1 mm in length; observations were made at 24-h intervals for 10 days. One set of treatment consisted of 50 seeds with 4 replications. After each count, the germinated seeds were discarded.

Effect of seed size and acid scarification period followed by priming with PEG-6000 on seed germination

Three-g samples of 3 seed sizes were soaked in 50 mL of sulfuric acid at room temperature from one to three hours, depending on the seed sizes. The seeds were rinsed thoroughly with tap water and then primed for 7 days, by soaking in 30 mL of 30% (w/w) PEG-6000 (-1.3 MPa) solution at 10℃. The seeds were rinsed again with tap water and germination tests were conducted on moistened filter paper for 10 days. One set of treatments consisted of 50 seeds with 3 or 4 replications, as above.

Effect of seed priming with sea water and PEG-6000 after acid scarification on seed germination

Medium-sized, scarified seeds were used to compare the effect of sea water with 30% PEG-6000 as seed priming agents. Seed lots of 300 seeds each were scarified in 100 mL 18 N H_2SO_4 for two hours at room temperature. The seeds were rinsed thoroughly with tap water; subsequently they were soaked for 1 week in 100 mL of one of the following solutions; 30% PEG-6000, and sea water at 3 different concentrations of full (-2.5 MPa), 2/3 and 1/2 strength. Seeds of the control lot were soaked in water for 2 h (described in Fig. 1 as water control). After priming, all seeds were stored for one week (or 2 weeks for the non-primed lots) in desiccators with silica gel at room temperature. A germination test consisting of 100 seeds was triplicated. Seeds that began to germinate during priming with 1/2 strength -seawater were eliminated before the germination test at 30℃.

Effect of dehydration after primings with sea water or NaCl following acid scarification on seed germination

Three hundred medium-sized seeds that were scarified as above, were rinsed and primed with either 30% PEG-6000, 1.5% and 3.0% (-2.2 MPa) NaCl, or full strength sea water for 1 week at 10℃; acid scarified seeds or water-steeped seeds served as well. All seeds were stored for 10 months in desiccators with silica gel at room temperature. A germination test was carried out in triplicate as above.

Results

Effect of recycling sulfuric acid solution as a scarifying agent on seed germination

The use of 18 N H_2SO_4 resulted in 50% germination within 5 to 6 days; no non-scarified seeds germinated (Table 1). Final germination percentages resulting from the acid treatment ranged from 50 to 62%, compared to 18% when soaked in tap water.

Although the volume of the acid solution decreased from the initial 100 mL to a final volume of 88 mL, recycling the acid four times did not significantly affect its scarification efficiency. Scarification in 36 N H_2SO_4 for a shorter period effectively promoted seed germination, but the acid solution could not be re-used because of high viscosity that resulted from contamination with organic matters dissolved from the pericarp (data not shown).

Effect of seed size and acid scarification period followed by priming with PEG-6000 on seed germination

The test on seed size versus scarification period on germination (Table 2) revealed that the largest seeds required a longer scarification period to attain the same germination percentage. For small-sized seeds, an hour of scarification was sufficient to promote germination without any injury; 1.5 and 3 h treatments resulted in slight and severe injuries, respectively.

Medium and large-sized seeds required scarification period of 2 and 3 h, respectively for faster and higher germination rates. The final percentage after 10 days of incubation was above 80% in the small- and medium-sized seeds, whereas that of large seeds varied signifi-

Table 1. Spinach seed germination at high temperature (30℃) as affected by acid scarification with re-used sulfuric acid.

Treatment[z]	Days to 50% germination	Final germination (%)[y]
1st	6.1	50 ± 2.3
2nd	5.8	53 ± 1.9
3rd	5.2	62 ± 3.5
4th	5.4	58 ± 2.6
Cont.	not-attained	18 ± 1.1

[z] Medium sized seed population classified in weight of 1.2 g per 100 seeds, was selected and scarified for 2 h successively in each lot at an 18 N H_2SO_4.

[y] Final germination percentage ± SE (n=4) was evaluated 10 days after incubation.

Table 2. Effect of spinach seed size on germination after treatment with sulfuric acid followed by PEG-6000 priming.

Seed size[z]	Duration of scarification (min)	Days to 50% germination	Final germination (%)
Small	60	2.4	82 ± 3.2
	90	2.1	61 ± 2.9[y]
	120	died	died
Medium	60	3.8	74 ± 2.5
	90	3.3	73 ± 3.6
	120	2.5	84 ± 4.1
Large	90	not-attained	42 ± 4.9
	120	7.1	52 ± 3.4
	180	3.9	63 ± 2.3

[z] Populations of small, medium or large seeds were defined by 0.6 g, 1.2 g and 2.7 g as a weight per 100 seeds, respectively.
[y] A few non-germinated seeds died from acid injury.

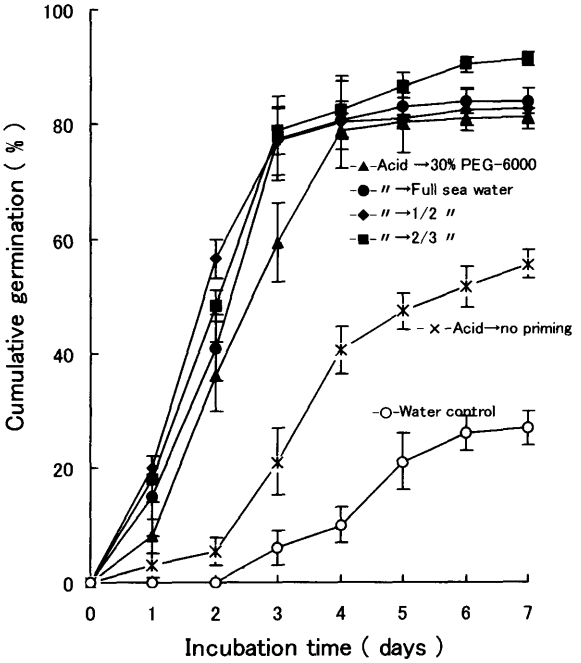

Fig. 1. Cumulative germination percentage of spinach seeds at 30℃ as affected by acid scarification, followed by priming with PEG, NaCl, or sea water. Vertical bars represent SE (n = 3 or 4).

cantly with the duration of acid scarification; the germination percentage increased when the soaking period was extended.

Effect of seed priming with sea water and PEG-6000 following acid scarification on seed germination

Priming with different dilutions of sea water and 30% PEG-6000 following acid scarification had little effect of the final germination (Fig. 1). In a preliminary

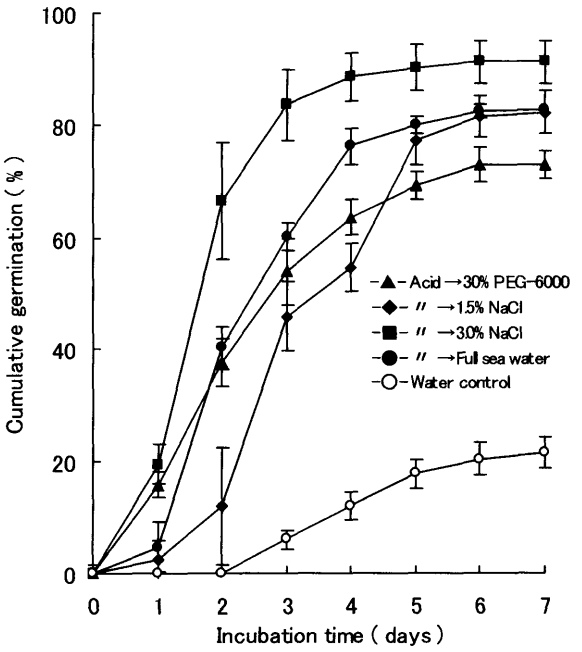

Fig. 2. Cumulative germination percentage of dehydrated spinach seeds after a 10-month storage following acid scarification and priming with PEG, NaCl, or sea water. Vertical bars represent SE (n = 3).

experiment, treatment of full strength of sea water or with NaCl was not as effective as in PEG-6000 alone (data not shown) as observed by Masuda and Konishi (1993).

Effect of seed dehydration after priming with sea water or NaCl following acid scarification on seed germination

Effect of dehydrated seeds after priming with sea water or NaCl solution following acid scarification on subsequent seed germination showed that priming with 1.5% NaCl, sea water and PEG-6000 significantly enhanced the germination percentage even after 10-month storage at room temperature (Fig. 2). A few seeds that germinated in 1.5% NaCl solution during the priming were eliminated from the germination test. The germination pattern did not differ among the treatments of 30% PEG-6000, 1.5% solution of NaCl and full strength of sea water. At the end of 7-day incubation period, the highest germination rate was obtained in 3% NaCl, followed by 1.5% NaCl, the full strength of sea water, and 30% PEG-6000, respectively.

Discussion

Seed germination of spinach was inhibited markedly at temperatures above 25℃ (Masuda and Konishi, 1993). Seed priming has been established for many kinds of seeds, but the optimum condition for spinach seed priming was -1.25 MPa water potential of 30% PEG-6000 at 10℃ (Atherton and Farooque, 1983b). The single treatment with PEG-6000 was not sufficient to

enhance the germination rate of spinach, but an acid scarification treatment prior to the priming was significantly promoted it (Masuda and Konishi, 1993). Our data suggest that the use of 18 N H_2SO_4 up to 3 times maintained its potential of promoting seed germination. The remaining re-used solution seemed to be effective in seed priming until it was entirely used up. The duration required for soaking in the acid, however, depended on the seed size; the larger seeds required a longer soaking time to achieve high germination rates (Table 2). This indicated that spinach seeds should be graded according to size, prior to any such scarification treatment; a scarification period greater than 3 h may be advantageous for the large-sized seeds. Although large-sized seeds do not germinate readily at high temperatures, the seedlings established from larger seeds are more vigorous than those from smaller ones. The germination percentage of PEG-treated spinach seeds did not change when air-dried and stored for 7 or 14 days (Nakamura et al., 1982). The improved germination of primed spinach seeds was retained after 30 days of storage at 5℃ (Atherton and Farooque, 1983b). The $NaOCl/H_2O_2$ pre-sowing seed treatment yielded the higher germination rate at 30℃, compared to that of seeds without their pericarp because of the occurrence of atypical seedlings (Katzman et. al., 2001). However, their report did not elucidate on the best seed scarification methods that would enhance spinach seed germination with normal, vigorous seedlings. Our results demonstrate that priming with full strength of sea water, 3% NaCl, and 30% PEG-6000 enhanced dehydrated spinach seed germination even after 10-month in storage. This procedure is very important as it allows for post priming transport of seeds and alleviates the germination problems encountered by growers because the seed merchants can prime the seeds prior to distribution to growers.

Although PEG-6000 or 8000 is commonly used as seed priming agent, inorganic salts, such as KNO_3, KH_2PO_4, K_3PO_4 and $CaCl_2$, are also commonly used (Bradford, 1986; Haigh and Barlow, 1987; Yoon et al., 1997). Undiluted or diluted sea water would be an inexpensive alternative to technical or reagent grade salts or PEG, especially in developing countries, provided sea water is available. In this experiment, sea water and NaCl solution were just as effective as PEG for promoting germination of spinach seeds (Figs. 1 and 2). There are some reports in a comparison of priming agents for salt tolerant asparagus seeds and moderately salt sensitive tomato seeds (Frett et al., 1991) which indicate that synthetic sea water, at about 1/3 strength was effective or better than PEG for tomato seeds. Our study clearly shows that scarification with 18 N H_2SO_4, followed by soaking in a 2/3 or a full strength of sea water solution prevents seeds from germination during the priming and subsequent storage (Fig. 2). This protocol may be a cheap and effective method of seed pre-treatment to enhance spinach germination during the hot summer months. Seemingly, the use of sea water as a priming agent is of no hindrance for germination, since spinach seeds have a tolerance to high salinity.

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ホウレンソウの高温30℃での種子発芽に及ぼす酸処理および塩水と海水のプライミング処理ならびに種子再乾燥処理の影響

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摘　要

ホウレンソウ (*Spinacia oleracea* L.) は高温で発芽が抑制される．18 N 硫酸溶液で種子処理すると30℃において発芽率は著しく向上した．硫酸溶液を4回連続使用しても種子の発芽促進効果は低下しなかった．硫酸の最適処理時間は，種子の大小によって異なった．硫酸処理後，10℃で1週間PEG-6000，NaClあるいは海水に浸漬すると，発芽は早まり最終発芽率は硫酸単独処理に比べてさらに向上した．硫酸処理後，3% NaCl溶液でプライミング処理を行ったあと10か月乾燥貯蔵しても，種子は30℃で80%以上発芽し，貯蔵前に認められた高い発芽力は消失しなかった．海水も同様に高いプライミング効果を有し，PEG-6000あるいはNaCl溶液に代替させることが可能であり，ホウレンソウ種子へのプライミング法として夏季の高温時において，とくに有効であると考えられた．