Seed Enhancements to Improve Spinach Germination

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Additional index words. Spinacia oleracea, decoating, dormancy, germination inhibitors, hydrogen peroxide, sodium hypochlorite, hydroponics, temperature

Abstract. Rapid, synchronized, and high percentage of germination is required for commercial spinach (Spinacia oleracea L.) production using hydroponic techniques. Seed treatments examined to improve seed germination were: 1) decoating; 2) leaching in water; and 3) soaking seeds for 4 hours in 0.5% NaOCl, leaching for 15 hours in water, and sowing in 0.3% H2O2 (this treatment will be referred to as NaOCl/H2O2). Germination studies were conducted on four cultivars at a constant 18°C (optimal) or 30°C (inhibitory). At 18°C, germination rate (T50) was maximized by both hydration treatments, but uniformity of germination (Tsd) was greatest for decoated seeds; final germination was ≥89% for all treatments. At 30°C, decoating resulted in greatest uniformity of germination. The NaOCl/H2O2 treatment resulted in highest germination (94%) at the high temperature, whereas decoating was least effective (69%). Reduced germination of decoated seeds was attributed to atypical germinants. Cultivars differed in response to the treatments at both temperatures. Component analysis of the NaOCl/H2O2 treatment was studied with two slow-to-germinate cultivars. Treatment with H2O2 or without NaOCl improved the rate, uniformity, and percentage of germination of seeds of both cultivars, but NaOCl alone did not. Percipcar removal or pericarp removal plus NaOCl/H2O2 treatments reduced variability in germination time and enhanced speed of germination at 30°C, but decoating produced a higher percentage of atypical seedlings than did other treatments. Therefore, the NaOCl/H2O2 treatment is recommended for growers who are unable to maintain cool germination temperatures and/or cannot afford the costs associated with cooling. If growers can maintain a germination temperature of ≈18°C, decoated seeds are preferable, based on the high uniformity of germination.

Spinach is a challenging crop to germinate and grow in a hydroponic system. Seed dormancy may cause poor germination and variable plant stands. Nongerminating seeds waste expensive space and resources in the growth chamber or greenhouse. Speed and uniformity of germination are also important, since slow-to-germinate seeds and poor uniformity of germination result in uneven growth. Presowing hydration or physical treatments may be useful for enhancing germination.

The spinach “seed” is botanically a fruit with a spirally coiled embryo surrounding a pocket of nonliving tissue called the perisperm, but the term “seed” will be used throughout this paper (Taylor, 1997). Orientation of the embryo is peripheral, and it is covered by the pericarp.

Exogenous and endogenous factors may be responsible for germination inhibition or dormancy in spinach (Katzman, 1999). Germination declines progressively at temperatures >20°C. Dormancy has been attributed to chemical inhibitors and/or physical properties of the pericarp (outer fruit coat), as pericarp removal permits germination at high temperatures. The pericarp may reduce germination by physically blocking radicle emergence and/or limiting the amount of oxygen available to the embryo for respiration.

Seed treatments may overcome dormancy and enhance germination by altering the physical integrity of seed coverings, allowing the seed to complete early phases of germination under more favorable conditions, or both. Seed enhancements are postharvest treatments that improve germination and seedling growth, or facilitate the delivery of seeds and other materials required at the time of sowing (Taylor et al., 1998). Seed enhancements for spinach include removal of the pericarp, hydration, or chemical treatment of imbibing seeds. Decoating improves germination and extends the range of temperatures at which spinach seeds can germinate (Atherton and Farooque, 1983; Leskovar and Esensee, 1999; Suganuma and Ohno, 1984). Soaking in NaOCl and H2O2 can also stimulate germination by weakening the seed covering tissues, enhancing gas exchange, or a combination of both (Katzman, 1999; Ku et al., 1996).

Chemical inhibitors in the seed coverings may also reduce germination. Germination of spinach seeds was improved at 30°C by leaching the seeds to remove water-soluble inhibitors (Atherton and Farooque, 1983). Seeds imbibed with this leachate germinated poorly in comparison with those imbibed in water. Oxidants such as H2O2 and NaOCl may oxidize inhibitors within the seed coat (Bewley and Black, 1982). In addition, sodium hypochlorite can kill fungi and bacteria on the seed surface and thereby enhance seedling root and shoot growth (Chun et al., 1997).

Heydecker et al. (1969) suggested that high temperatures might inhibit spinach seed germination by reducing the amount of dissolved oxygen in water, while increasing embryo respiration rate and metabolic demand. Therefore, another method of enhancing germination is exposure of imbibed seeds to oxygen levels higher than that of air, e.g., exposure to pure oxygen or to oxygen-liberating compounds such as H2O2 (Heydecker et al., 1969). Catalase, which is present in seeds, converts H2O2 to oxygen and water (Bewley and Black, 1982). Collectively, favorable conditions for spinach seed germination involve cool temperature (18 to 20°C) and high availability of oxygen.

The main goal of this study was to improve the percentage, rate, and uniformity of germination of spinach cultivars selected for a commercial hydroponic production facility. To achieve this goal, the effects of selected seed treatments (decoating, leaching, and a NaOCl/H2O2 sequence of chemical and hydration treatments) were evaluated at both 18 and 30°C. The NaOCl/H2O2 presowing seed treatment was applied to slow-to-germinate cultivars, and selected steps were examined independently to determine their effects.

Materials and Methods

Seed sources. Smooth leaf Japanese cultivars Okame, Megaton, Alrite, and Dash are used in commercial hydroponic production in Japan. Seeds of these cultivars were provided by Takii Seed Co. (Kyoto, Japan) and commercially prepared as both decoated and intact seeds. Semi-savoy leaf American cultivars Rushmore and Whitney were obtained from Alf Christianson Seed Co. (Mount Vernon, Wash.). These cultivars were selected based upon growth characteristics, such as high yields and tolerance to bolting.

Experimental procedures. Seeds were placed on blue blotter papers in acrylic germi-
nation boxes (11.2 × 11.2 × 3.5 cm high) (Hoffman Manufacturing Co., Albany, Ore.). Each box contained 50 seeds evenly spaced on two layers of blotter paper, which was moistened with 15 mL of reverse osmosis (RO) water or 0.3% H₂O₂ solution, depending on treatment. No additional solution was supplied to the seeds during the experiments. A seed was considered germinated if 1 mm of radicle had emerged through the pericarp. Atypical germination occurred when the cotyledons emerged before the radicle; these atypical seedlings were counted as germinated if and when the radicle emerged. The number of germinated seeds was counted at 24-h intervals for a period of 5 d after initial sowing. Day zero was designated as the day on which the seeds were sown. Each 50-seed germination box was replicated four times for a total of 200 seeds per treatment.

**The effects of presowing seed treatments on germination at 18 °C and 30 °C.** Seeds, provided by Takii Seed Co. (which utilizes a proprietary technology for removing the pericarp), were treated and then placed on two blotters in germination boxes with 15 mL reverse RO or 0.3% H₂O₂ solution depending upon presowing treatment. Treatments were as follows: 1) intact seeds were sown in RO water (control); 2) decoated seeds were sown in RO water; 3) intact seeds were soaked in 2 L of vigorously aerated RO water for 19 h and sown in RO water (leached); and 4) intact seeds were soaked in 2 L of 0.5% NaOCl for 4 h, then in 2 L of vigorously aerated RO water for 15 h, then sown in 0.3% H₂O₂. Treatment 4) will be referred to as NaOCl/H₂O₂. Bleach with a labeled concentration of 5.25% NaOCl was diluted 1:9 with water. The 0.3% H₂O₂ solution was obtained by diluting a labeled 3% U.S.P. solution of H₂O₂ to 1:9 with water.

**Component analysis of the NaOCl/H₂O₂ treatment at 18 °C.** Selected steps of the NaOCl/H₂O₂ treatment were examined independently to determine their effects on germination of seeds of the slow-to-germinate cultivars, Rushmore and Whitney. The solutions of NaOCl and H₂O₂ were prepared as previously described. Seeds were treated prior to germination at 18 °C as follows: 1) leaching (as described above); 2) NaOCl (same as NaOCl/H₂O₂, above, except that seeds were sown in RO water); 3) H₂O₂ (same as leached, except that seeds were sown in 0.3% H₂O₂); and 4) NaOCl/H₂O₂ (as described above).

**Data analysis.** Germination counts were recorded daily for the first 5 d after sowing. From these counts, two other measures of germination were derived: speed of germination and uniformity of germination. Germination curves from the daily germination counts were plotted. Speed of germination, T₅₀, was the number of days required for half the seeds to germinate; uniformity of germination, Tₛₐ, was the number of days required for one standard deviation of the total number of germinants to germinate (as described by Hacisalihoğlu et al., 1999). The T₅₀ and Tₛₐ were obtained for each repetition of each treatment by performing a standard nonlinear curve fit using the ordinary logistic model of GENSTAT (Hacisalihoğlu et al., 1999). Data were analyzed as a 2-way (cultivar × seed treatment) analysis of variance for each dependent variable (T₅₀, Tₛₐ, and percentage of germination on day 5) for all experiments, and Fisher’s LSD was used to compare treatment means. Means with standard errors were calculated for the germination of normal versus atypical seedlings at 30 °C.

**Results and Discussion**

The effects of presowing seed treatments on germination at 18 °C. In each cultivar studied, T₅₀ was highest for the control treatment, followed by the decoated treatment, while the hydration treatments resulted in the fastest germination (Table 1). However, cultivar differences in response resulted in significant interaction. This acceleration was attributed to the prehydration phase, since the leached and NaOCl/H₂O₂ treated seeds were imbibed for 19 h, whereas control and decoated seeds were not hydrated prior to germination.

Decoated seeds had the lowest mean Tₛₐ value, while ‘Megaton’ showed little response to any treatment (Table 1). This suggests that alteration or removal of the pericarp may be an important source of seed-to-seed variation in time required for germination. The final percentage germination on day 5 was generally 29% for all four cultivars. However, the nontreated ‘Okame’ and decoated ‘Dash’ had slightly lower germination (91% and 89%, respectively).

The effects of presowing seed treatments on germination at 30 °C. NaOCl/H₂O₂-treated seeds had the fastest germination at 30 °C followed by increasingly higher T₅₀ values for decoated, leached, and control seeds (Table 1). Decoating generally resulted in the lowest Tₛₐ values and final percentage of germination (average 69%). Atypical germinants ranged from 15% to 35% for the four seed lots, and were responsible for the reduced normal germination (Table 2). ‘Megaton’ had the lowest percentage of atypical seedlings, and therefore this seed lot was least sensitive to high temperature. Percentage of germination was 29% when the data for normal and atypical seedlings were combined.

The NaOCl/H₂O₂ treatment consistently induced the highest final percentage of germination of all the presowing seed treatments at 30 °C, and may have counteracted the effects of inhibitors in the pericarp that are responsible for thermosensitivity. In support of this hypothesis, Hsiao and Quick (1984) showed that NaOCl and H₂O₂ break dormancy in wild oat (Avena fatua L.) seeds by modification of the seed coverings. In addition to counteracting chemical inhibitors, H₂O₂ increases availability of oxygen to seeds at high temperatures by providing supplemental oxygen for respi...
Table 2. Effects of decoating seeds on percentage germination of four spinach cultivars after 5 d at 30 °C.

| Cultivar | Normal germinants (mean ± se) | Atypical germinants (mean ± se) | Total (mean ± se) |
|----------|------------------------------|--------------------------------|------------------|
| Dash     | 60 ± 3                        | 35 ± 3                         | 95 ± 4           |
| Atrite   | 70 ± 1                        | 27 ± 2                         | 97 ± 3           |
| Okame    | 65 ± 6                        | 29 ± 1                         | 94 ± 2           |
| Megaton  | 83 ± 1                        | 15 ± 1                         | 98 ± 2           |

Table 3. Component analysis of the effects of NaOCl/H2O2 pre-sowing seed treatment with NaOCl and H2O2, alone and together, on germination of two spinach cultivars at 18 °C.

| Cultivar | Treatment | T50 (d) Mean ± se | Tsd (d) Mean ± se | Germination (%) Mean ± se |
|----------|-----------|-------------------|-------------------|---------------------------|
| Rushmore | Water     | 3.4 ± 1           | 2.6 ± 1           | 62 ± 1                    |
|          | NaOCl     | 2.6 ± 1           | 2.2 ± 1           | 70 ± 1                    |
|          | H2O2      | 2.0 ± 1           | 1.4 ± 1           | 90 ± 1                    |
|          | NaOCl/H2O2| 1.8 ± 1           | 1.2 ± 1           | 92 ± 1                    |
|          | Whitney   | 4.4 ± 1           | 2.7 ± 1           | 86 ± 1                    |
|          | NaOCl     | 3.6 ± 1           | 2.6 ± 1           | 74 ± 1                    |
|          | H2O2      | 2.4 ± 1           | 2.0 ± 1           | 94 ± 1                    |
|          | NaOCl/H2O2| 1.9 ± 1           | 0.9 ± 1           | 100 ± 1                   |

**Interaction LSD**

| Treatment | T50 (d) Mean ± se | Tsd (d) Mean ± se | Germination (%) Mean ± se |
|-----------|-------------------|-------------------|---------------------------|
| Water     | 3.9 ± 1           | 2.7 ± 1           | 74 ± 1                    |
| NaOCl     | 3.1 ± 1           | 2.4 ± 1           | 72 ± 1                    |
| H2O2      | 2.2 ± 1           | 1.7 ± 1           | 91 ± 1                    |
| NaOCl/H2O2| 1.9 ± 1           | 1.0 ± 1           | 96 ± 1                    |

**Cultivar**

- **Rushmore**: T50 = 2.4 ± 1, Tsd = 1.6 ± 1, Germination = 92 ± 1
- **Whitney**: T50 = 2.4 ± 1, Tsd = 1.8 ± 1, Germination = 79 ± 1

**Significance**

- **Cultivar**: NS
- **Treatment**: NS
- **Cultivar × treatment**: NS

Mean separation within columns and sets by Fisher’s LSD test, *P ≤ 0.05. Nonsignificant or significant at 0.05.

**Component analysis of the NaOCl/H2O2 treatment at 18 °C.** Selected steps of the NaOCl/H2O2 treatment were examined for their effects on germination in a 2 × 4 (two cultivars × four treatments) factorial experiment. The main effects of cultivar and treatment were significant for T50, while only treatment affected Tsd. Interaction was significant for final percentage of germination, but not for T50 and Tsd (Table 3).

This experiment was performed at 18 °C, since most hydroponic growth facilities are maintained at 18 °C, decoated seeds are recommended. They consistently had the most uniform germination and were convenient to use, since no additional time or effort was required to apply the treatment. Also, decoated seeds can be sown dry with an automatic seeder, and no posttreatment drying procedure is required. Thus, for the most uniform germination in a hydroponic system, sowing decoated seeds is recommended.

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