Germination and fungal infection of wild celery (*Apium graveolens* L.) seeds, from southern Brazil, under different temperature and disinfection conditions

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ABSTRACT

Seeds of wild celery (*Apium graveolens* L.) from southern Brazil were surface disinfected with different solutions of sodium hypochlorite (5 and 10%) and acetic acid (0.5, 1, 2, 4%), and germination success and fungal infection were evaluated after 28 days of incubation at a constant temperature of 30 ºC and 20/30 ºC thermoperiod (12h:12h). Germination of wild celery was inhibited at the constant temperature (30 ºC). Vigorous total germination (90–100%), a faster germination velocity (1.8–2.5 germinated seeds per day) and moderate fungal infection (53.3–81.7%) of wild celery seeds were obtained with the sodium hypochlorite treatments (5–10% concentration) under the 20/30 ºC thermoperiod. The 4% treatment of acetic acid was very effective at preventing seed fungal infection (only 5% of the seeds) but it reduced the average total germination to 60%. Lower concentrations of acetic acid (0.5–2%) resulted in 100% fungal infection. In conclusion, seedlings of wild celery from southern Brazil can be effectively produced by disinfecting the seeds with 5–10% sodium hypochlorite and incubation under a 20/30 ºC thermoperiod (12h:12h).

Keywords: acetic acid; fungi; sodium hypochlorite; prophylaxis.

INTRODUCTION

Apium graveolens (Apiaceae) is an herbaceous marshland plant commonly used for consumption since antiquity, mainly due to its unique taste, nutritional composition, fiber content and innumerous pharmaceutical uses (Yao *et al*., 2010; Shad *et al*., 2011; Uddin *et al*., 2015). Browers & Orton (1986) stated that *A. graveolens* is distributed in coastal marshes of Eastern Europe, Asia Minor, North Africa and North America, and that three botanical varieties of celery (*i.e*., var. *dulce*, *rapaceum* and *secalinum*) were domesticated (also called smallage and marsh parsley). Nowadays, several cultivar varieties (cvs.) of celery are found worldwide (Yao *et al*., 2010; Uddin *et al*., 2015).

Wild celery varieties have an increasing market interest and horticultural value, due to their growth behavior (*i.e*., elongated) and peculiar flavor (*i.e*., pungent acrid) (Browers & Orton, 1986; Yao *et al*., 2010), since customers of gourmet foods are more open to new varieties of edible vegetables. Some *A. graveolens* varieties have a marked salt tolerance (*i.e*., halophytes) inherited from their ancestors that inhabited salt marshes (Everard, 1994), and cultivating these varieties with irrigated salt or brackish water can be marketed as environmentally friendly due to fresh water conservation, which increases the value of these vegetables. In southern Brazil, Costa (1997) recorded a biannual halophytic variety of *A. graveolens* that occurs in salt marshes of the Patos Lagoon estuary. This wild variety has free phenolic compounds content (known bioactive compounds) that is 10-fold greater than values found in commercial celery cultivars (Souza *et al*., 2018). However, initial studies about the domestication of this halophytic variety had a major setback due to intense seed

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infestation by fungi during germination. In Brazil, main fungal diseases recorded for commercial *A. graveolens* var. *dulce* are produced by seedborn fungi *Rhizoctonia solani*, *Pythium* spp., *Phytophthora nicotianae* and *Alternaria dauci* (Reis *et al*., 2018). According to Silveira (2012) *Rhizoctonia* sp. is one of most frequent fungi occurring on leaves of salt marsh plants of Patos Lagoon estuary during summer-autumn, but no proper identification of fungi on seeds of the halophytic variety of *A. graveolens* was done.

According to Coolbear *et al*. (1992), *A. graveolens* is known for being difficult in relation to germination and establishment. Temperature and photoperiod can influence the germination of *A. graveolens*, and there are different responses among the varieties (Thompson, 1974). Seed germination of this species usually takes a long time and there is some asynchrony under suboptimal temperatures (Van der Toorn & Karssen, 1992). The pericarp of celery seeds has alternating longitudinal furrows (yellowish parts) and ridges (darkish parts), and schizogenous oil tubes run beneath the furrows (Hopkins, 1927). Volatile oil obtained from celery seeds is used in the perfume and pharmaceutical industries (Shad *et al*., 2011), but the presence of ridges and oil tubes on the seed pericarp make it difficult to clean the seeds, favoring the development of fungi during germination. Surface seed disinfection by germicide application is necessary to remove microorganisms that may interfere with germination (Abdul-Baki & Moore, 1979).

Among the many alternatives, sodium hypochlorite and acetic acid are the most affordable and easy to find, because they are used domestically as household bleach and vinegar, respectively. Sodium hypochlorite (NaOCl) is a chemical typically used as a sterilizing agent of seeds, since it does not affect seed germination and seedling growth (Abdul-Baki & Moore, 1979). Acetic acid (CH_3COOH) is an organic acid used as a seed disinfectant and adopted by organic agriculture, since it has low eco-toxicological risk (Van der Wolf *et al*., 2008). Besides the need to control seed infestation by microorganisms, exposure time and concentration of a disinfectant can affect seed germination and may lead to losses in viability. Thus, the establishment of a proper disinfectant procedure for seeds can improve germination percentage and the successful establishment of *A. graveolens* plantlets. The aim of the present study was to evaluate applications of sodium hypochlorite and acetic acid as disinfectants and to determine the best temperature for germination of the halophytic wild variety of celery (*A. graveolens*) found in southern Brazil.

MATERIAL AND METHODS

Material

Seeds of the halophytic wild variety of *A. graveolens* were collected in the Pólvora Island salt marsh located in the Patos Lagoon estuary, Rio Grande, RS (32º01'S; 52º06'W). The seeds were dried at room temperature (20/25 ºC) for 30 days and then stored at 5 ºC in the germplasm bank of the Laboratório de Biotecnologia de Halófitas (Instituto de Oceanografia – IO, Universidade Federal do Rio Grande – FURG) for six months before the experiments.

Experiment 1. Temperature effect on germination and fungal infestation

Seeds were surface sterilized by soaking them for 5 minutes using three disinfection solutions: 5% and 10% sodium hypochlorite, and 4% acetic acid. The concentrations of sodium hypochlorite were made from a dilution of 2.5% active chlorine (common concentration of Brazilian household bleach) and the concentration of acetic acid was made from Brazilian vinegar (4% acetic acid; Brazil, 2000); they were prepared with pure chemicals. After disinfection, the seeds were rinsed with distilled water and placed in autoclaved Petri dishes with filter paper dampened with 6 mL of distilled water. The Petri dishes were placed in germination chambers at a constant temperature of 30 ºC and thermoperiod of 20/30 ºC. Seed incubation lasted for 28 days and both chambers had a photoperiod of 12 h light/12 h dark (40 µmol photons $m^{-2}s^{-1}$, 400–700 nm; provided by cold white fluorescent light). Three Petri dishes with 20 seeds were used as replicates of each treatment. Seed germination (radicle protrusion) was recorded every week and the percentage of seeds with fungal infestation after one week of incubation was used as a proxy of disinfection efficiency. For this procedure individual seeds were graded according to a 2 – digit pathogenicity scale (0 and 1); whereby 0 indicates without fungi, 1 = with fungi. Due to the high fungal infestation at the end of first week, the disinfection treatments were once repeated.

Experiment 2. Disinfection procedure effect on germination and fungal infestation

Due to the strong inhibition of seed germination by the 4% acetic acid solution, three additional disinfection treatments with lower concentrations of acetic acid (0.5%, 1% and 2%) were tested in a second 28-day trial using only the 20/30 ºC thermoperiod. This second experiment used the same photoperiod, number of seeds per Petri dish and number of replicates as the first experiment.

Statistical analysis

Germination speed index (GSI) was calculated as described in Maguire (1962) and expressed in germinated seeds per day. Average values of total germination percentage and percentage of seeds with fungal infection were compared between temperatures (20/30 ºC and 30 ºC)

and among disinfection treatments (5% and 10% sodium hypochlorite, and 4% acetic acid) using a bifactorial ANOVA. Data for the disinfection treatments of both germination trials exposed to the thermoperiod were combined and the total germination percentage, GSI and percentage of seeds with fungal infection were compared with a one-way analysis of variance (ANOVA). Germination speed index values for the constant temperature (30 ºC) were not calculated due to the absence of germination under this experimental condition. The requirements of normality and homoscedasticity for the ANOVA procedures (Zar, 2010) were evaluated using Shapiro-Wilk's and Levene's tests, respectively. Significant differences in the ANOVA ($p < 0.05$) were followed by Fisher's least difference (LSD) test at 5% significance.

RESULTS AND DISCUSSION

The germination and seed fungal infection data are in Tables 1 and 2. At a constant temperature of 30 ºC (Table 1), no germination of the wild celery seeds occurred after 28 days of incubation. In contrast, at the 20/30 ºC thermoperiod up to 100% of the seeds germinated when they were disinfected with sodium hypochlorite. Previously, Morinaga (1926) found a maximum germination for *A. graveolens* (cvs. Dreers Monarch and Columbia) between 50–70% at a 22/32 ºC thermoperiod. Thompson (1974) also reported that a thermoperiod of 22/25 ºC was most effective for the germination of other cultivar varieties of *A. graveolens* (cvs. Golden Self-blanching, Avon Pearl, Lathom blanching, Giant Red and Solid White), but he noted that germination velocity response to temperature can be different for each celery variety. Concerning the effect of constant temperatures, Hopkins (1927) worked with cultivar varieties of celery and Parera *et al*. (1993) studied non-primed *A. graveolens* seeds (cv. M-68-29-5) and found overall seed germination percentages of 28% and 2% at 30 ºC, respectively. Coolbear *et al*. (1992), working with pre-imbibed seeds of two cultivars of *A. graveolens* (cvs. Tall Utah 52-70 and Green Giant Hybrid), recorded only an average of 6% germination of seeds exposed to 25 ºC for 34 days. Morinaga (1926) observed no germination for varieties of *A. graveolens* at 32 ºC for 30 days. According to Biddington *et al*. (1980), a high temperature (32 ºC) may induce secondary dormancy of *A. graveolens* seeds (cv. Lathom Blanching), possibly preventing embryo development and endosperm breakdown, making the seed deal directly or indirectly (pre-imbibed and dried seeds) with desiccation.

Fungal disinfection of wild *A. graveolens* seeds was statistically better for the 4% acetic acid treatment (lowest fungal infection = 5% of the seeds), but this procedure strongly inhibits the average total germination (60% at the 20/30 ºC thermoperiod after 28 days). Lowering the concentration of acetic acid (0.5–2%) led to fungal infection of 100% of the seeds, lower germination velocities and smaller final total germination than seeds disinfected with sodium hypochlorite (Table 2). Similarly, Van der Wolf *et al*. (2008) found a marked decrease in disinfection efficiency of acetic acid on seed-associated bacteria with this dilution. According to Doran (1929), acetic acid may have a toxic effect on higher plants. For the sodium hypochlorite treatments, 53.3–81.7% of seeds were infected by fungi after one week of incubation, but the seeds showed high germination velocities (average GSI =1.8–2.5 germinated seeds per day) and final total germination values above 90%. Pathogenicity scale did not distinguish several levels of infestation (only infected and not infected seeds), but the results suggest that sodium hypochlorite treatments were effective to inhibit fungi seed damage, and the most concentrated solution allowed the highest

Table 1: Mean ± (standard error) of the total germination and fungal infection of the wild *A. graveolens* under thermoperiod and constant temperature among disinfection treatments. Summary of two-way ANOVA results for all parameters among disinfection levels and seed incubation temperatures are presented

Treatment	Total germination (%)		Fungal infection (%)	
	20/30 °C [$]	30 °C	20/30 °C	30 °C
5% NaOCl	90.0 c (5.8)	0.0 a (0.0)	53.3 b (8.8)	68.2 bc (5.6)
10% NaOCl	100.0 d (0.0)	0.0 a (0.0)	81.7 c (10.9)	65.2 bc (2.2)
4% CH_3COOH	60.0 b (2.9)	0.0 a (0.0)	5.0 a (2.9)	6.1 a (1.5)
F Disinfection	31.2 (***)		46.1 (***)	
F Temperature	1500.0 (***)		0.00 (ns)	
F Disinfection x Temperature	31.2 (***)		1.2 (ns)	

[$] Different lowercase letters represent significant differences between temperatures and disinfection treatments, according to Fisher's least difference (LSD) test at 5% significance. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns: non-significant ($p > 0.05$).

Table 2: Mean ± (standard error) of the total germination, germination speed index (GSI) and fungal infection of the wild *A. graveolens* under thermoperiod among disinfection treatments. Summary of one-way ANOVA results for all parameters among disinfection treatments are presented

Treatment	Total germination (%) [$]	GSI (Germinated seeds per day)	Fungal infection (%)
5% NaOCl	90.0 c (5.8)	1.8 ab (0.2)	53.3 b (8.8)
10% NaOCl	100.0 c (0.0)	2.5 c (0.0)	81.7 c (10.9)
0.5% CH_3COOH	75.0 b (2.9)	2.1 bc (0.1)	100.0 d (0.0)
1% CH_3COOH	68.3 ab (1.7)	1.7 ab (0.2)	100.0 d (0.0)
2% CH_3COOH	70.0 ab (7.6)	1.4 a (0.2)	100.0 d (0.0)
4% CH_3COOH	60.0 a (2.9)	1.6 a (0.2)	5.0 a (2.9)
F	12.0 (***)	5.2 (**)	42.5 (***)

[$] Different lowercase letters (within a column) represent significant differences between disinfection treatments, according to Fisher's least difference (LSD) test at 5% significance. *p < 0.05; **p < 0.01; ***p < 0.001; ns: non-significant (p > 0.05).

total germination. Taylor (1949) and Abdul-Baki & Moore (1979) pointed out that *A. graveolens* cultivar varieties responded well to concentrated sodium hypochlorite solutions, being the total germination of *A. graveolens* cv. Detroit Golden reduced somewhat by the solutions of 1.5% and 2% "active chlorine" tolerated by cv. Tall Utah (Taylor, 1949).

CONCLUSIONS

Seedlings of wild celery can be effectively produced by disinfecting the seeds with 5–10% sodium hypochlorite and incubating them under a 20/30 ºC thermoperiod (12h:12h).

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