Hypochlorite Solution Enhances the Seed Germination of *Echinacea angustifolia*

In-Jung Kim[1,3], In Sup So[2,3*]

[1]*Faculty of Biotechnology, College of Applied Life Sciences, Jeju National University, Jeju 690-756, Korea*
[2]*Department of Horticulture, Graduate School, Jeju National University, Jeju 690-756, Korea*
[3]*Research Institute for Subtropical Agriculture and Biotechnology, Jeju National University, Jeju 690-756, Korea*

Abstract

Narrow-leafed purple coneflower (*Echinacea angustifolia*) has been increasingly used as an herb in recent year. Establishment of this plant in the field for commercial production has been a challenge mainly due to difficulty in uniform seed germination. In this study, seed soaked in 10% household bleach (Clorox, 0.525% sodium hypochlorite) solution for 120 minutes showed a high percent germination (80%).

Introduction

Coneflower species (the genus *Echinacea*), native to the Great Plains and Midwest of the USA, have been used for medicinal purposes by indigenous Americans (Smith-Jochum and Albrecht, 1987; Li, 1998). Extracts from roots possess antibiotic and antiviral properties and stimulate immunological systems (Li, 1998; Kochankov et al, 1998). It is so valuable ornamentally for wild flower garden that many nursery have developed new cultivars every year. *E. angustifolia*, the narrow-leafed purple coneflower of the western Great Plains, is distributed on dry prairies from Texas to Saskachewan and from west of the Rocky Mountains to Minnesota (Feghahati and Reese, 1994). The two most popular species in the US and Canada are *E. purpurea* and *E. angustifolia*, and the latter has higher market value.

Smith-Jochum and Abrecht (1987) reported that *E. purpurea* exhibited significantly higher germination than *E. angustifolia* in the greenhouse. They were able to obtain only 50% germination of *E. angustifolia* achenes using a 15-min hypochlorite treatment followed by water soaking or stratifying for 1 or 2 months. It was much reported that stratification improved some wild flower seed germination. However *E. purpurea* seed germination enhanced a little bit more from 89% in control to 99% by stratification for 2-4 weeks and the longer period was not good for germination (Bratcher et al, 1993). Smith-Jochum and Albrecht (1987) concluded that stratification did not enhance seed germination of *E. purpurea* in the greenhouse. Thus we might propose *Echinacea* species don't need stratification to germinate.

In the last few years, supplies of *Echinacea* collected from wild plants have not met increased demand in Europe and North

America. Thus commercial cultivation is increasing rapidly and massive plug production system is needed. However information on germination, seedling growth and their effect on growth, yield and chemical composition is very limited. The objective of this experiment was to establish effective germination method of *E. angustifolia*.

Materials and Methods

Seed collection

Seeds of *E. angustifolia* were collected at Astro-farms (3009, Lystra Rd., Chapel Hill, NC 27514). Some of the seeds were preserved in refrigerator at 5 °C for 12 weeks and the others were in room temperature for same period. The stratified and non-stratified seed were mailed to North Dakota State University (Fargo, ND58105) for germination test.

Germination test

After seeds were treated with stratification, hulling, and bleaching, the seeds were sowed. The germination percentage was analyzed according days after sowing and at root formation stage (Table 1).

Table 1. Germination percentage of *Echinacea angustifolia* seeds according to treatments at days after sowing.

Seed treatment		Days after sowing				Root formation (%)
		3	5	7	Final	
Stratification	Un-hulled	35.6	43.6	47.5	47.5	41.7
	Hulled	12.3	13.5	13.5	13.5	0
Non-stratification	Un-hulled	41.0	56.1	58.8	58.8	53.7
	Hulled	9.0	11.5	11.5	11.5	0
Stratification		ns	ns	ns	ns	ns
Hulling		***	***	***	***	***
Stratification x hulling		ns	ns	ns	ns	ns

Bleaching treatment period test

Commercial household bleaching solution (Clorox, 0.525% sodium hypochlorite) was used to disinfect contaminants on the seed surface and stimulate germination. For selecting optimal treatment period, the stratified and non-stratified seed were soaked in 10% bleaching solution for 30, 60, 90, 120, 150 or 180 minutes. After soaking, the seed were dried in paper towel and then placed in germination plate. Germination was evaluated using 30-35 seeds placed in 100×15 mm petri dishes containing two layer of Whatman #2 filter paper and 5 ml sterilzed distilled water. Petri dishes were placed under two cool-white fluorescent lamps (800 μmol) in cultivation room at 25 °C. Each treatment was replicated four times.

Pathogen culture on PDA medium

To test disinfection effect on seed of *E. angustifolia* (stratified or non-stratified seeds), thirty seeds were treated with 15 ml bleaching solution (10% Clorox) for 60 minutes. After bleaching solution treatment, the seeds were rinsed with distilled water three times. The seeds were immersed into 15 ml autoclaved distilled water for 60 minutes. As control treatment, the other thirty seeds were put into 30 ml flask containing 15 ml autoclaved distilled water for 60 minutes. Six droplets per plate were inoculated onto half-strengthen PDA (Difco Co.) medium in a 100×15 mm petri dish (Falcon Co.). Three plate per treatment were replicated in this experiment.

Data collecting and statistical analysis

Every germinated seed (radicle emerged from seed hull) was counted daily for petri dish test and the cumulative total of germination was recorded for 7-10 days. The percent germination and standard error was calculated

from collecting data from four replication in each treatment. Seedling growth from emergence test was measured 12 days after seed sowing. Analysis of variance (ANOVA) and Duncan's multiple range test were performed on percent seed germination using Costat (CoHort Software, Minneapolis, MN) statistical package program.

Results and Discussion

In preliminary experiment, we found that chilling treatment alone at 5°C (seed stratification) did not seem to improve germination significantly and stratified seeds were contaminated 2-3 times more than non-stratified seed (unpublished data).

Too many kind of fungi and bacteria covered around seeds and inhibited germination. Household bleaching solution (0.5%, 5-10 minutes) has been commonly used for surface disinfection by commercial growers. Application of 10% bleaching solution was an effective means not only disinfecting the seed but also stimulating germination (Table 1 & Fig. 1B). In stratified or non-stratified seeds, the percent germination were improved from 40-50% in control to above 80% as the soaking period in bleaching solution increased up to 120-150 minute (Fig. 1). As the soaking period increased, the germination difference between stratified and non-stratified seed was decreased. It means that pre-chilling or stratification doesn't need to stimulate germination of *E. angustifolia* seed. Prechill alone did not improve seed germination of *E. angustifolia* (Feghhati and Reese, 1994). The germination of seed treated with bleaching solution over 150 minutes, was not good and it may attribute to a toxic effect of hypochlorite.

In terrestrial orchid, bleaching solution (both sodium hypochlorite and calcium hypochlorite) was reported to make crack on the seed surface which could absorb water easily (Miyoshi and Mii, 1998). Haynes et al (1997) proposed the acid and sodium hypochlorite treatment may increase gas exchange and water uptake into the switchgrass seeds through the corroded distal margin of the lemma. Hsiao and Quick (1984) suggested that the promotive effect of NaOCl on germination of wild oats may have resulted from increased

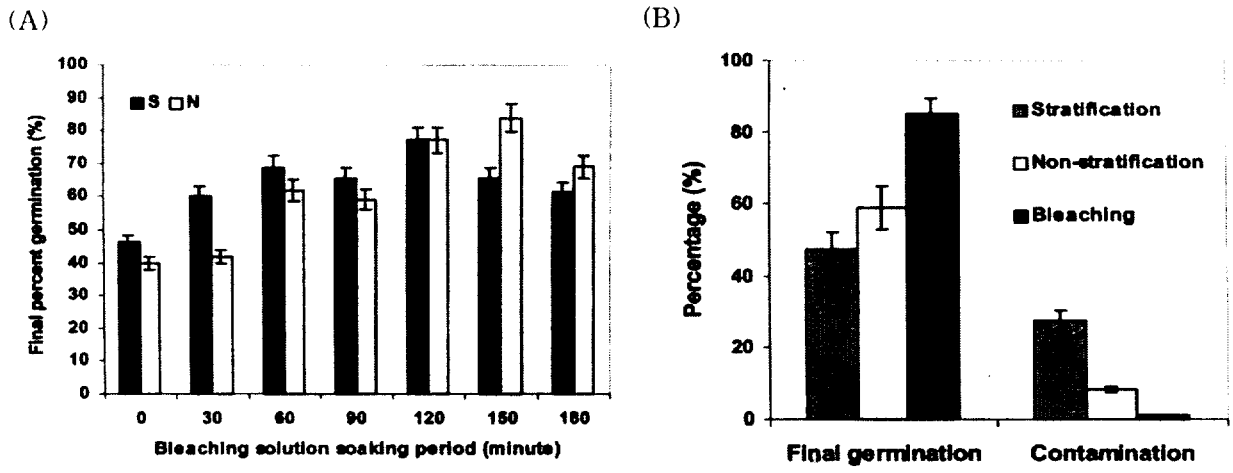

Fig. 1. Germination rate during bleaching solution soaking period (A) and percentages of final germination and contamination according to seed treatment (B). S means stratification and N is non-stratification.

sensitivity of the embryo to endogenous gibberellins, or the destruction of germination inhibitors. These results proposed that bleaching solution may be a germination-promoting material for seed of *Echinacea*. Its optimal concentration and treatment period might be various according to species, maturity, or storage condition.

References

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