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Short communication

Seed germination of a newly discovered hyperaccumulator *Solanum nigrum* L. affected by illumination and seed-soaking reagent

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ABSTRACT

Solanum nigrum is a newly found Cd-hyperaccumulator which showed very high remediation efficiency in polluted soil. Seed germination experiments with different illumination and seed-soaking reagents were conducted in constant temperature box and greenhouse with soil as burgeon base. The results showed that the germination rate with alternating light/dark photoperiod was about twice of that without lighting ($p < 0.05$), suggesting that illumination is one of the important conditions for seed germination of *S. nigrum*. All treatments with seed-soaking reagents significantly increased the seed germination rate of *S. nigrum* ($p < 0.05$). Treatment with H_2O_2 (0.1%) had the shortest germination time. The germination rate of seeds that were not washed in water following soaking was 2–3 times higher than that of seeds that were washed after soaking.

1. Introduction

Phytoextraction is widely regarded as a promising remediation technology with many advantages, such as low cost, no soil-structure destruction, no secondary pollution, environmental beautification, and easy acceptance by the people [1]. Phytoextraction is the use of hyperaccumulators to accumulate metals from soils by plant roots and the translocation/accumulation (phytoextraction) of contaminants into plant shoots until metal concentrations in the soil decrease to acceptable levels [1–3]. Although great progress has been made in the identification of hyperaccumulators, phytoextraction technology has not widely been used in remediation practice as yet. The main restricting factor is the low remediation efficiency of hyperaccumulators due to small biomass and long growing seasons [4,5].

Solanum nigrum is a newly found Cd-hyperaccumulator with higher biomass [4]. In a former published article, stems and leaves were discovered to be the main Cd hyperaccumulating organs of *S. nigrum*. The Cd extraction efficiency of *S. nigrum* by harvesting the shoot biomass at its flowering stage twice a year increased by 75.0% as compared to that at its single maturity [5]. Thus, a fast reproducible technology for reducing its growth time and for the application of two-phase planting method of phytoextraction is indispensable. In this study, the effects of illumination and seed-soaking treatments on seed germination of *S. nigrum* were evaluated to explore quick germination methods.

2. Materials and methods

2.1. Reagent selection and seed collection

Though some culture techniques aimed to obtain high yield and growth of *S. nigrum* have been reported, studies on expediting seed germination are still limited [6–8]. Reagents like, H_2O_2, KNO_3 and gibberellin that effectively promoted the germination in some plants have been used previously as seed based soaking reagents [9–12]. Therefore, these reagents were selected to determine their effects on the germination of *S. nigrum* seeds.

Seeds of *S. nigrum* were collected at its maturity (September 2008) from tree fields in Shenyang Station of Experimental Ecology, Chinese Academy of Sciences (41°31′ N and 123°41′ E). These were

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Table 1
Treatment levels of three tested seed-soaking reagents.

Level	H_2O_2 (%)	Gibberellin (mg L^{-1})	KNO_3 (%)
1	0.1	100	0.1
2	0.2	200	0.2
3	0.3	300	0.3

stored in a paper bag at room temperature (22–25 °C) until further use (3 months after collection).

2.2. Effect of illumination on germination

Some collected seeds were sterilized by using 1% NaOCl for 10 min, followed by washing with tap water, and air drying [9,10]. In order to simulate local farmland germination environment, soil collected from the station was used as burgeon base. The soil was topsoil (0–20 cm) of meadow burozem with pH 6.65 and 1.5% organic matter, which was sieved through 1 mm sieve before use.

Fifty seeds soaked in distilled water for 12 h at 30 °C in a constant temperature box without lighting were evenly laid on the soil surface in petri dishes (15 cm in diameter) and covered with a soil layer about 3 mm thick [9,10]. Supplement by distilled water using sucker was done every day to sustain soil humidity [11,12]. Four replicates of unsealed dishes were placed in the constant temperature box in dark condition, and another four replicates were placed in a greenhouse at 25 °C either with alternating light/dark (10/14 h) environment. Fluorescent lamps were used to produce a photosynthetic photon flux density of 400 μmol/m^2 s.

Germination rates (%) were recorded every day up to the 10th day when the germination ratio was no higher than 1% in five consecutive days [13,14].

2.3. Seeds soaked germination experiment without washing prior to showing

Three different concentrations of each reagent (Table 1) were used according to some published articles, i.e. some effective concentrations on plant seed germination, including 0.2% H_2O_2 for capsicum seed [9], 0.2% KNO_3 for capsicum seed and cotton seed [10,11] and 200 mg L^{-1} for Medicago sativa seed [12]. Seeds of *S. nigrum* were soaked in H_2O_2, KNO_3, and gibberellins (AR, produced by Kermel company) for 12 h at 30 °C in the constant temperature box without lighting. Then 50 seeds soaked in each of these solutions were directly sown per dish without washing by distilled water using the same method as mentioned in Section 2.2. All dishes were kept in the greenhouse after irrigation. The treatments of seeds unsoaked and soaked with distilled water served as controls. Every treatment was replicated four times and the temperature and light environment in the greenhouse were the same as mentioned in Section 2.2. Germination rates (%) were recorded every day up to the 10th day [13,14].

2.4. Seed-soaked germination experiment with washing prior to showing

The method described in Section 2.3 was followed. The difference between the two experiments was that all seeds soaked in three reagents were washed by using distilled water before sowing. Germination rates (%) were recorded every day up to the 10th day [13,14].

2.5. Data analyses

The average and standard deviation (SD) of four replicates for each treatment were calculated using Microsoft Excel software and analysis of variance was performed at $p < 0.05$ using SPSS 13.0 software [15].

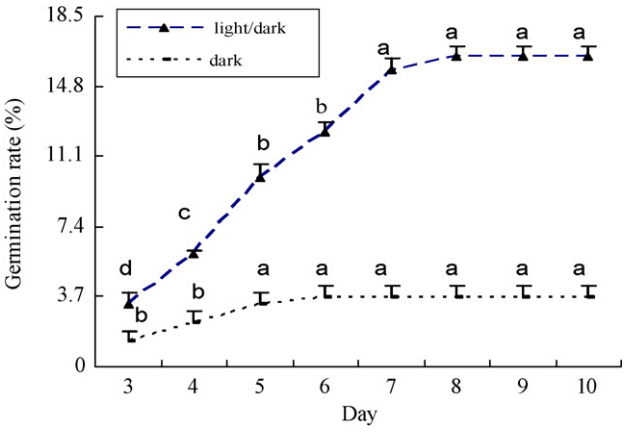

Fig. 1. Germination rate (%) of *S. nigrum* seed soaked in distilled water. The difference between two treatments with different letters in the same curve is significant ($p < 0.05$).

3. Results and discussion

3.1. Effect of illumination on the seed germination of S. nigrum

As shown in Fig. 1, the germination rates of *S. nigrum* seeds soaked in distilled water significantly increased ($p < 0.05$) with time between 3 and 5 days under dark condition and between 3 and 6 days in the alternating light/dark condition. However, their germination ratios did not show an increase after the 5th day of the former and the 6th day of the latter treatments. The highest germination rate was only 16.3%, which is very low (germination rate <80%) [13,16]. Thus, reagent soaking may be indispensable for better germination of *S. nigrum* seeds.

In dark environment, the rates of seed germination on the 5th day and the 10th day were 3.3 % and 3.7 %, respectively (Fig. 1). However, under the alternating light/dark condition, the germination ratios were 3.7% and 16.3%, respectively. The latter was about twice as high as of the former, suggesting that light is one of important primary factors in the seed germination of *S. nigrum*.

Some reports of seed germination responses to light are inconsistent. Hawton and Drennan reported greater germination of 1-year-old seeds in light/dark (40%) than in the dark (21%) [17]. However, Ismail et al. found 100% germination of plant seeds under darkness [18]. This shows that seed germinations of different plants have varying responses to light. Light may stimulate seed germination of many plants, especially in plants with small seeds such as *Hibiscus trionum*, *Atriplex patula*, *Leptochloa chinensis* and *Eleusine indica* [19–21]. Because small seeds have limited amount of food reserves, better germination and emerging may occur only when seeds are exposed to light at or near the soil surface. Thus, the role of light in stimulation of germination in *S. nigrum* seeds could be because of their relatively small size.

3.2. Effect of reagents on the germination of S. nigrum seeds sown without washing

When the seeds were soaked in different reagents without washing prior to sowing, germination rates were significantly increased ($p < 0.05$) (about 2–8 times higher than that of unsoaked and 1–4 times higher than that in distilled water on the 10th day), indicating that soaking reagents were effective in improving *S. nigrum* seed germination (Table 2). Some research results showed that gibberellin contains abscisic acid (ABA). H_2O_2 is

Table 2
Germination rate of *S. nigrum* seeds without cleaning after soaking in different reagents (%).

Treatment		Treatment time (days)							
Reagent	Level	3	4	5	6	7	8	9	10
Unsoaked		0.3 ± 0.6c	2.7 ± 0.6f	4.3 ± 0.6f	6.3 ± 1.2g	6.7 ± 1.5g	9.3 ± 0.6g	9.3 ± 0.6f	9.3 ± 0.6f
Distilled water		3.3 ± 0.6b	6.0 ± 1.0e	10.0 ± 1.0e	12.3 ± 0.6f	15.7 ± 0.6f	16.3 ± 0.6f	16.3 ± 0.6e	16.3 ± 0.6e
H_2O_2	0.1%	9.0 ± 2.0a	21.0 ± 2.0b	87.7 ± 4.2a	96.7 ± 1.5a	97.0 ± 2.0a	97.7 ± 1.5a	98.3 ± 1.5a	98.3 ± 1.5a
	0.2%	9.7 ± 1.2a	18.7 ± 4.2bc	90.0 ± 4.0a	98.0 ± 1.0a	98.3 ± 1.2a	98.3 ± 1.2a	98.3 ± 1.2a	98.3 ± 1.2a
	0.3%	10.0 ± 1.0a	17.0 ± 2.0bc	93.7 ± 2.1a	97.3 ± 1.2a	97.3 ± 1.2a	98.8 ± 1.5a	99.3 ± 0.6a	99.3 ± 0.6a
KNO_3	0.1%	8.7 ± 1.2a	18.0 ± 2.0bc	55.3 ± 3.1b	65.0 ± 2.6c	75.3 ± 2.3c	90.0 ± 2.0b	94.0 ± 3.6a	94.0 ± 3.6a
	0.2%	10.0 ± 2.0a	16.0 ± 2.0c	56.3 ± 5.0b	65.7 ± 2.9c	77.3 ± 2.3c	90.7 ± 2.3b	95.3 ± 4.6a	95.3 ± 4.6a
	0.3%	11.0 ± 1.5a	27.3 ± 2.4a	61.3 ± 3.1b	82.0 ± 2.0b	88.0 ± 3.5b	91.0 ± 3.5b	98.0 ± 2.0a	98.0 ± 2.0a
Gibberellin	100 mg L^{-1}	8.0 ± 1.0a	13.3 ± 2.3d	17.0 ± 1.0d	19.7 ± 1.5e	20.3 ± 2.1e	24.0 ± 2.0e	31.0 ± 1.0d	35.7 ± 2.1d
	200 mg L^{-1}	9.3 ± 0.6a	18.0 ± 2.0bc	25.0 ± 2.0c	32.7 ± 1.5d	40.7 ± 3.1d	49.7 ± 1.5d	54.3 ± 3.1c	64.7 ± 1.5c
	300 mg L^{-1}	8.3 ± 1.2a	16.0 ± 1.0c	20.0 ± 3.6cd	33.0 ± 1.0d	41.7 ± 2.5d	62.0 ± 2.0c	66.7 ± 3.1b	75.0 ± 2.6b

Note: The difference between two treatments with different letters in the same column is significant ($p < 0.05$).

Table 3
Germination rate of *S. nigrum* seeds with cleaning after soaking in different reagents (%).

Treatment		Treatment time (days)							
Reagent	Level	3	4	5	6	7	8	9	10
Unsoaked		0.3 ± 0.6b	2.7 ± 0.6c	4.3 ± 0.6e	6.3 ± 1.2d	6.7 ± 1.5f	9.3 ± 0.6f	9.3 ± 0.6g	9.3 ± 0.6i
Distilled water		3.3 ± 0.6a	6.0 ± 1.0b	10.0 ± 1.0d	12.3 ± 0.6c	15.7 ± 0.6d	16.3 ± 0.6d	16.3 ± 0.6f	16.3 ± 0.6g
H_2O_2	0.1%	4.3 ± 0.6a	11.3 ± 1.5a	24.3 ± 1.2c	28.0 ± 2.0b	34.7 ± 3.1b	39.7 ± 3.1b	42.7 ± 1.2c	45.3 ± 1.2c
	0.2%	5.3 ± 1.5a	13.7 ± 1.2a	30.0 ± 2.0b	35.0 ± 2.0ab	40.3 ± 4.5ab	42.3 ± 3.1a	47.3 ± 2.1b	50.7 ± 0.6b
	0.3%	5.7 ± 1.2a	14.3 ± 1.5a	36.7 ± 1.2a	39.2 ± 2.0a	42.7 ± 4.0a	48.4 ± 3.1a	54.0 ± 1.5a	57.6 ± 0.6a
KNO_3	0.1%	4.0 ± 1.0a	11.7 ± 1.2a	20.0 ± 4.0cd	30.7 ± 2.3b	33.0 ± 2.0ab	36.3 ± 2.3b	37.0 ± 2.0cd	37.0 ± 2.0de
	0.2%	4.7 ± 0.6a	12.7 ± 0.6a	25.0 ± 1.7c	30.3 ± 2.3b	33.7 ± 1.2ab	37.3 ± 0.6b	39.0 ± 1.0cd	39.3 ± 1.2d
	0.3%	5.3 ± 1.5a	14.3 ± 1.5a	25.3 ± 2.5c	30.3 ± 2.5b	34.7 ± 3.1ab	38.7 ± 1.5b	40.3 ± 1.5cd	42.0 ± 1.0d
Gibberellin	100 mg L^{-1}	4.0 ± 1.0a	7.3 ± 1.2b	11.0 ± 1.0d	12.7 ± 0.6c	12.7 ± 1.2e	13.3 ± 0.6e	13.7 ± 0.6f	14.3 ± 0.6h
	200 mg L^{-1}	4.3 ± 1.2a	7.7 ± 1.5b	14.3 ± 1.5d	16.0 ± 1.0c	19.7 ± 2.1c	21.3 ± 2.5c	23.0 ± 3.0e	25.7 ± 4.0f
	300 mg L^{-1}	4.7 ± 0.6a	8.3 ± 2.1b	11.7 ± 2.9d	15.0 ± 2.0c	21.7 ± 2.1c	32.7 ± 2.5b	33.7 ± 2.5d	34.0 ± 2.0e

Note: The difference between two treatments with different letters in the same column is significant ($p < 0.05$).

a strong oxidant, and K^+ in KNO_3 is an activator of oxidative enzymes [9–12]. Thus, these reagents can promote seed germination to some extent by improving O_2 scavenging capacity, increasing certain activity in enzyme, or by repairing destroyed seed cell membrane.

The germination rates of seeds soaked in various concentration of H_2O_2 on the 5th day were 87.7%, 90.0% and 93.7%, respectively, and had the shortest germination time [13,16]. Though the germination rate of seeds soaked in KNO_3 was also higher than 80% (82.0%) on the 7th day the germination time was longer. As for the germination rates of seeds treated by gibberellins, all ratios were lower than 80%, even on the 10th day (35.7%, 64.7% and 75.0% for three concentrations, respectively). Thus, H_2O_2 was a better seed-soaking reagent among the three reagents used and 0.1% level was sufficient in bringing about a significant increase in germination rate. In addition, all seedlings treated by reagents were healthy as compared to the controls, which also suggested that these soaking reagents were effective in seed germination in *S. nigrum*.

3.3. Effect of reagents on the germination of S. nigrum seeds sown after washing

Although germination rates of *S. nigrum* seeds soaked with all three reagents and then washing with distilled water before sowing were significantly ($p < 0.05$) higher than the controls (seeds without soaked and soaked by distilled water) (Table 3), their germination rates were not higher than 80%, which germinated not quite well [16]. Compared to the treatments of seeds soaked in the three reagents without washing before sowing, germination rates when the seeds were not washed prior to sowing were about 2–3 times that when the seeds were washed prior to sowing ($p < 0.05$), suggesting the effects of the former is better (Tables 2 and 3). It may be caused by the quick soaking method (only 12 h). The reagents conglutinated on the surface of seeds may be still effective in seed germination of *S. nigrum* even though the soaking treatment has been over [13].

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