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Germination and dormancy of seeds in *Echinacea purpurea* (L.) Moench (Asteraceae)

P. LI[1], H. WU[1]*, S. GENG[1], X. WANG[1], W. LU[2], Y. YANG[1], L.M. SHULTZ[3], T. TANG[1] AND N. ZHANG[4]

[1] Center for Medicinal Plant Research, South China Agricultural University, Guangzhou 510642, China (E-mail: wh@scau.edu.cn)
[2] Guangdong Key Lab for Post Harvest Science, South China Agricultural University, Guangzhou, 510642, China
[3] College of Natural Resources, Utah State University, Logan, UT 84322-5230, USA
[4] Bureau of Science and Technology of Guangzhou Municipality, Guangzhou 510091, China

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Summary

Seeds of *Echinacea purpurea* (L.) Moench display low germination rates and a wide variation in rates of emergence, hindering production considerably. A few factors, all known to affect germination of *Echinacea purpurea* seeds were investigated. It was found that treatments of gibberellic acid (GA_3) and ethephon significantly increased germination percentage and shortened mean germination time (MGT) and prechilling treatment resulted in a large increase in percentage and rate of germination, both in darkness and in light. A prechilling treatment for 14 days at 4°C in a 200 mg/L GA_3 or 1 mM ethepon solution in continuous light, followed by a germination period in light at 20/30°C (16/8h), induced over 90% seed germination. In addition, removing the seed coats or damaging the seeds also proved to be conducive to improving seed germination. The experimental results showed that dormancy of *E. purpurea* seeds is partly due to seed coat restriction. The dormancy mechanisms are discussed.

Introduction

Echinacea purpurea, commonly referred as purple coneflower, is one of the nine *Echinacea* species (Asteraceae) native to North America (McGregor, 1968). *E. purpurea* has been shown to possess antibiotic and antiviral effects and may stimulate the immune system (Kindscher, 1989; Reith, 1978; Wacker and Hilbig, 1978). Because of it's important medicinal value, this species has been introduced to many countries, such as Germany, Spain, Australia and New Zealand. There are nine *Echinacea* species, three of which, *E. purpurea*, *E. angustifolia* and *E. pallida*, have been used as medicinal plants.

Propagation of *E. purpurea* by seed is limited by embryo dormancy (Smith-Jochum and Albrecht, 1987), with seed lots displaying low germination rates and a wide variation in emergence rates (Altwater, 1980). Chemical and physical methods have been used to address these problems. GA_3 treatments have either promoted seed germination (Macchia *et al.*, 2001; Smith-Jochum and Albrecht, 1987) or had no effects (Duan *et al.*, 2004;

* Author for correspondence

Kochankov *et al.,* 1998) on *E. purpurea* seeds. Several members of the Asteraceae family, including *E. purpurea* and *E. angustifolia,* have been shown to require ethylene to break seed dormancy (Abeles and Lonski, 1969; Corbineau and Côme, 1987; Feghahati and Reese, 1994; Katonh, 1975; Kochankov *et al.,* 1998; Jones, 1968; Smith-Jochum and Albrecht, 1987). However, Pill and Haynes (1996) found ethephon during priming of *E. purpurea* seeds did not promote seed germination. Undoubtedly, prechilling can promote germination, but the treatment time varied with temperature and seed source (Abeles and Lonski, 1969; Baskin *et al.,* 1992; Carlma *et al.,* 1993; Parmenter *et al.,* 1996). Light has either promoted seed germination (Feghahati and Reese, 1994) or had no effect (Wartidindingsih and Geneve, 1994) on *E. purpurea* seeds. Little is known about the mechanism of seed dormancy of *E. purpurea.* The pericarp does not affect seed germination of *E. angustifolia* (Feghahati and Reese, 1994), but the seed coat restricted germination (Duan *et al.,* 2004), the reason for which has not been reported.

The objectives of this study were to establish an effective method for improving seed germination. While our interest is in expanding the production of *E. purpurea* grown in South China (Guangzhou), the results so far obtained are applicable to production in other parts of the world. In this study, we analyzed the relationships between seed coat and dormancy and between damaging seeds and promoting germination. Possible mechanisms for *E. purpurea* seed dormancy are also discussed.

Materials and methods

E. purpurea (only one population) was cultivated at the farm of South China Agricultural University (Guangzhou). Heads were randomly chosen from different plants and bulked to provide a uniform source of seeds for use in germination studies. Mature seeds of *E. purpurea* were collected from plants after scapes had dried but before achenes were dispersed on August. Seeds were cleaned and impurity was removed with the help of forceps. The mean length of seed was 5.07±0.30mm (n=50), the mean width of seed was 2.28±0.12mm (n=50), the mean weight of seed was 4.09±0.16mg (n=1000). Dry seeds were stored hermetically for 3 months at room temperature prior to germination testing. The climate of Guangzhou (22°35′ - 23°35′N, 112°57′-114°03′E) is subtropical with the average annual temperature of 21.4-21.9°C. The highest temperature is 38.7°C while the lowest is –2.6°C; The average annual rainfall is 1624 to 1900mm; the average annual solar radiation is 4367 to 4597MJ/m^2.

Each treatment had three replications of 50 seeds (with an additional 50 seeds in experiment 9, 10 and 11). All seeds were soaked in 1% NaClO solution for 15 minutes and rinsed with distilled water three times. The surface sterilized seeds were evenly placed on two layers of filter paper and moistened with distilled water or growth regulator in glass Petri dishes (D=9cm). Germination when the radicle appeared was recorded daily [Association of Official Seed Analysts (AOSA), 1986]. Mean germination time (MGT) was calculated using the formula $\Sigma nd/N$ (n = numbers of germinated seeds on each day, d = number of days from the beginning of the test, N = total number of germinated seeds). Mean germination rate was calculated using the the formula 1/MGT. Germination percentages were normalized by transformation (arcsin $\sqrt{}$ %) before analyses of variance.

All experiments were arranged in completely randomized design.

1. Temperature and GA_3 treatment
Seeds of *E. purpurea* were treated with GA_3 at 0 (distilled water), 200, 500 or 800 mg/L and incubated at alternating of 20/30°C (16/8h) or constant 20°C in light [20 μmole $m^{-2}s^{-1}$photosynthetically active radiation (PAR)].

2. Temperature and ethephon treatment
The design of this experiment was similar to the initial experiment except that GA_3 was replaced by ethephon at 0 (distilled water), 1, 2 or 3 mM.

3. Light and prechilling treatment
Seeds were prechilled at 4°C in constant light (95μmole $m^{-2}s^{-1}$ PAR) or in the dark for 0, 7, 14 and 21 days. After the prechilling treatment, seeds were transferred to a germination chamber at 20/30°C (16/8h) in light (20 μmole $m^{-2}s^{-1}$ PAR).

4. Light, prechilling and GA_3 treatment
The seeds were placed on filter paper moistened with GA_3 solution at 0, 200, 500 or 800 mg/L in Petri dishes in light (95 μmole $m^{-2}s^{-1}$ PAR) or in the dark at 4°C. After 0, 7, 14 and 21 days' treatments, seeds were transferred to 20/30°C (16/8h) in light (20 μmole $m^{-2}s^{-1}$ PAR).

5. Light, prechilling and ethephon treatment
The design of this experiment was similar to the fourth experiment except that GA_3 was replaced by ethephon, at 0, 1, 2 or 3 mM.

6. Phosphoric acid, low pH and ethephon treatment
Seeds were treated with 1 mM ethephon solution, water adjusted to pH 3.8 with HCl to match the acidity of the ethephon, or 1 mM phosphoric acid individually and then placed in germination chamber at 20/30°C (16/8h) in light (20μmole $m^{-2}s^{-1}$ PAR).

7. Anatomical treatments
Using forceps, dissecting needle and razor blade, the seeds were manipulated to give the following: pericarp removed (T1); seed coat removed (T2); pericarp removed and the seed cut to divide longitudinally (T3); and the seed cut to divide the seed longitudinally followed by treating with 1 mM $Co(NO_3)_2$ (T4) (Co^{2+} is an enzyme inhibitor for endogenous C_2H_4). These treated seeds were then placed in germination chamber at 20/30°C (16/8h) in light (20 μmole $m^{-2}s^{-1}$ PAR).

8. Effects of mechanical resistance of seed coat
Seeds were treated according to the following operations: pericarp removed (CK), pericarp removed and treated with 50% H_2SO_4 for 5 minutes (Feghahati and Reese, 1994) (T1); seed coat removed only in the region of the radicle (T2); seed coat removed only in the region of the cotyledon (T3).

9. Measurement of water absorption ratio

One hundred seeds with or without seed coat were weighed and evenly placed on two layers of filter paper moistened with distilled water within glass Petri dishes. The treatments were placed in germination chamber at 20/30°C (16/8h) in light (20 μmole m^{-2} s^{-1} PAR). The seeds were dried with filter paper and weighed every 2 hours. Then seeds were placed in glass Petri dishes again and this process was repeated until seeds ceased absorbing water. Then seeds were dried and weighed and water absorption ratio of seeds was calculated (absorption water of 100 g dry seeds). This set of experiments follows the procedure described by Zhang *et al.* (2000).

10. Respiratory rate of E. purpurea seeds

One hundred seeds with or without seed coat were placed in airtight bottles and one milliliter of air was extracted with a syringe at 12 hours intervals and analyzed for CO_2 concentration using gas chromatography. Gas sampling proceeded until germination stabilized, 108 hours and 324 hours for seeds without and with seed coat respectively. The conditions for gas chromatography were: SHIMADZU GC-17A fitted with a 1.5 m×4 mm (internal diameter) stainless-steel column packed with alumina; injector temperature 120°C; column temperature 60°C; Flame ionisation detector (FID) temperature 120°C; Carrier gas (Helium) flow rate, 30 mL/min.

11. Measurement of endogenous ethylene produced by damaged E. purpurea seeds

One hundred seeds, without the seed coat and one hundred equally divided seeds, were placed in uncapped bottles for 2 hours (a preparative experiment established the optimal time for enough ethylene production). The bottles were capped and after 2 hours. One milliliter of air was extracted by syringe to detect the ethylene concentration. The gas chromatography conditions were similar to experiment 10.

Results

1. Temperature and GA_3 treatment

GA_3 (200-800mg/L) enhanced germination percentage and shortened MGT both at constant 20°C and alternating 20/30°C (16/8h), however, the concentrations of GA_3 had no effect at either 20°C or at 20/30°C. While temperature region had no effect on germination percentage, MGT was lowed by alternating 20/30°C (table 1).

2. Temperature and ethephon treatment

All ethephon concentration increased germination percentage except the 1 mM at 20°C and also shortened the MGT except 3 mM at 20/30°C (16/8h) (table 1).

3. Light and prechilling treatment

Prechilling significantly increased germination percentage (except the treatment of prechilling for 7 days in the dark) and rate of germination both in the dark and in light. No significant differences in germination rate were observed between prechilling for 14 days and 21 days, but prechilling for 14 days or 21 days increased the seed germination rate

significantly compared with prechilling for 7 days. Prechilling in the light was superior to prechilling in the dark (table 2).

4. Light, prechilling and GA_3 treatment

Significant increases in germination percentage and germination rate were observed for each GA_3 concentration prechilled in light or in the dark, however, there were no significant differences among treatments of different GA_3 concentrations. The germination percentage was higher in the light than in the dark for each GA_3 concentration and for each prechilling time. Significant increase in germination percentage and rate of germination were also observed for each GA_3 concentration when prechilling was extended from 7 to 14 days in light. The highest germination percentage and the highest germination rate can be obtained with 200 mg/L GA_3 and a prechilling period of 14 days in light (table 3). Our observation revealed that the interaction of light and prechilling significantly increased germination percentage and germination rate.

Table 1. Effects of temperature and GA_3 (ethephon) on seed germination of *E. purpurea.*

Treatments		Germination (%)			MGT (d)		
		20°C	20/30°C (16/8h)	mean	20°C	20/30°C (16/8h)	mean
GA_3 (mg/L)							
	0	63.3bA	62.0bA	62.7b	15.6aA	14.0aB	14.8a
	200	74.0aA	75.3aA	74.7a	11.7bA	10.3bB	11.0b
	500	72.7aA	71.3aA	72.0a	11.7bA	10.7bB	11.2b
	800	73.3aA	75.3aA	74.3a	12.0bA	11.3bA	11.7b
Ethephon (mM)							
	0	63.3bA	62.0bA	62.7b	15.6aA	14.0aB	14.8a
	1	72.0abA	74.7aA	73.4a	12.3bA	11.2bB	11.8b
	2	74.0aA	75.3aA	74.7a	11.3bA	10.1bB	10.7b
	3	77.3aA	76.0aA	76.7a	11.7bA	12.3abA	12.0b

Treatments followed by the same letter are not significantly different at the 0.05 probability level according to the LSD test (small letters in each column and capital letters in each row).

Table 2. Effects of light and prechilling (4°C) on seed germination of *E. purpurea.*

Duration of prechilling (d)	Germination (%)		MGT (d)	
	Light (24h)	Darkness	Light (24h)	Darkness
0	62cA	66.7 bA	14aA	14.3aA
7	74bA	71.3abA	9.3bB	11.7bA
14	82.7aA	76aB	6.3cB	8.3cA
21	79.3abA	75.3aB	7.3cA	8cA

Treatments followed by the same letter are not significantly different at the 0.05 probability level according to the LSD test (small letters in each column and capital letters in each row).

Table 3. Effects of light, prechilling and GA_3 on seed germination of *E. purpurea.*

Duration of prechilling (d)		GA_3 (mg/L)									
		Germination (%)					MGT (d)				
		0	200	500	800	Mean	0	200	500	800	Mean
Light	7	74.0bcB	82.7bA	84.7bcA	83.3bA	81.2bc	9.3bA	5.3bB	6.7bB	6.0bB	6.8b
	14	82.7aB	95.3aA	92.7aA	92.0aA	90.0a	6.3cA	3.7cB	4.0cB	3.7cB	4.4c
	21	79.3abB	90.0aA	87.3abA	92.7aA	87.3a	7.3cA	5.7bB	4.3cB	4.7bcB	5.5c
Darkness	7	71.3cB	79.3bA	80.7cA	80bA	77.8c	11.7aA	8.7aB	8.3aB	7.7aB	9.1a
	14	76.0bcB	85.3bA	87.3bA	83.3bA	83.0b	8.3bA	5.7bB	6.0bB	5.7bB	6.4b
	21	75.3cB	80.7bA	81.3bcA	79.3bA	79.1c	8.0bcA	6.0bB	5.3bB	5.3bB	6.2b

Treatments followed by the same letter are not significantly different at the 0.05 probability level according to the LSD test (small letters in each column and capital letters in each row).

5. Light, prechilling and ethephon treatment

Significant increases in germination percentage and germination rate were observed for each ethephon concentration prechilled in light or in the dark. But there were no significant differences among treatments of different ethephon concentrations. The germination percentage was higher in light than in the dark for each ethephon concentration and for each prechilling time. Significant increases in germination percentage and rate of germination were also observed for each ethephon concentration when prechilling was extended from 7 to 14 days. The highest germination percentage and the highest germination rate could be obtained with 2 mM ethephon and a prechilling period of 14 days in light (table 4). Our observation revealed that the interaction of light and prechilling as well as prechilling and ethephon significantly increased germination percentage.

6. Phosphoric acid, low pH and ethephon treatment

Ethylene and phosphate are both slowly released during the degradation of ethephon at low pH. Acidic conditions and either of the degradation products could contribute to the increase in seed germination. The increased germination percentage and rate of seeds treated with ethephon seemed to be due to the effects of ethylene, since low pH or phosphate alone had no effect on total germination percentage (data not shown).

7. Anatomical treatment

Removal of the pericarp did not influence the seed germination, while removal of the seed coat significantly promoted seed germination of *E. purpurea.* Removing the pericarp and cutting to equally divide the seed shortened MGT in comparison with removing seed coat and 1 mM $Co(NO_3)_2$ significantly restricted germination of seeds with removed pericarp and equally divided treatment (table 5).

Table 4. Effects of light, prechilling and ethephon on seed germination of *E. purpurea.*

Duration of prechilling (d)		Ethephon (mM)									
		Germination (%)					MGT (d)				
		0	1	2	3	Mean	0	1	2	3	Mean
Light	7	74.0bcB	84.0bA	86.0bA	81.3bA	81.3b	9.3bA	6.3bB	7.0bB	6.7bB	7.3b
	14	82.7aB	93.3aA	94.7aA	90.7aA	89.7a	6.3cA	4.0cB	4.3cB	3.7cB	4.6c
	21	79.3abB	90.7aA	89.3abA	94.7aA	88.5a	7.3cA	3.7cB	4.3cB	4.7bcB	5.0c
Darkness	7	71.3cB	79.3cA	78.6cA	81.3bA	77.6c	11.7aA	9.0aB	9.3aB	9.3aB	9.8a
	14	76.0bcB	86.7bA	85.3bA	84.0bA	83.0b	8.3bA	5.7bB	6.0bB	5.3bB	6.3b
	21	75.3cB	82.6cA	78.7cA	86.0bA	80.7b	8.0bcA	5.7bB	6.3bB	6.7bB	6.7b

Treatments followed by the same letter are not significantly different at the 0.05 probability level according to the LSD test (small letters in each compare in each column and capital letters compare in each row).

Table 5. Effects of anatomical treatments on seed germination of *E. purpurea.*

Treatments		Germination (%)	MGT (d)
CK	(with pericarp)	62.0c	14.2a
T1	(pericarp removed)	64.2c	13.7a
T2	(seed coat removed)	98.3a	4.5b
T3	(pericarp removed and equally divided longitudinally)	99.7a	2.7d
T4	(seed equally divided and treated with 1mM $Co(NO_3)_2$)	81.3b	3.4c

Treatments followed by the same letter in the same column are not significantly different at the 0.05 probability level according to the LSD test.

8. Effects of mechanical resistance of seed coat

No significant differences were observed among germination values (germination percentage and rate) recorded in seeds with only removed seed coat in the region of radicle and seeds with only removed seed coat in the region of cotyledon. The evidence indicated the seed coat did not mechanically restrict growth of the radicles. Furthermore, treatment with H_2SO_4 did not promote seed germination of *E. purpurea* (table 6).

9. Measurement of water absorption ratio

Removing the seed coat did not increase the ratio of water absorption for *E. purpurea* seeds (data not shown).

10. Measurement of respiratory rates

The respiratory rates of seeds remarkably increased when the seed coat was removed. In addition, removal of the seed coat enhanced the rate and percentage of germination (figure 1).

Table 6. Effects of mechanical resistance of seed coat on seed germination of *E. purpurea.*

Treatments		Germination (%)	MGT (d)
CK	(pericarp removed)	64.2b	13.7a
T1	(pericarp removed and treated with 50% H_2SO_4)	68.7b	12.8a
T2	(seed coat removed only covering the radicle)	98a	5.2b
T3	(seed coat removed only covering the cotyledon)	100a	4.9b

Treatments followed by the same letter in the same column are not significantly different at the 0.05 probability level according to the LSD test.

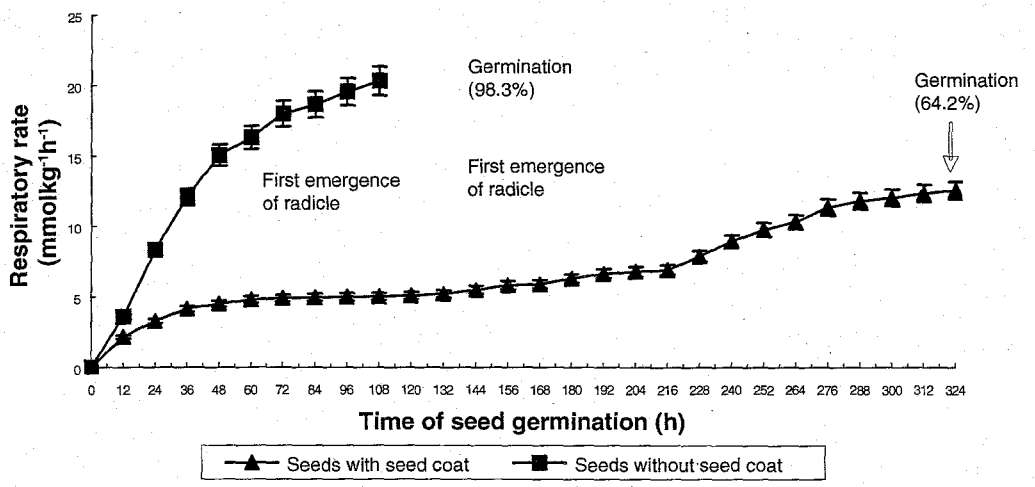

Figure 1. Effects of seed coat on seed respiratory rate of *E. purpurea.*

11. Measurement of endogenous ethylene produced by damaged E. purpurea seeds

There were no endogenous ethylene could be detected from seeds with seed coat removed. However, seeds with removed pericarp and equally divided, released endogenous ethylene at the rate of 0.685 $\mu lkg^{-1}h^{-1}$.

Discussion

1. GA_3 enhanced germination percentage and shortened MGT

GA_3 enhanced germination percentage and shortened MGT in comparison to controls both at 20°C or 20/30°C (16/8h). Smith-Jochum and Albrechet (1987) reported seeds of *E. purpurea* treated with either 1000 mg/L or 2000 mg/L GA_3 solution and had no effects on seed germination. Similar results were obtained in our study (data not shown). The reason for this may be because the concentrations of GA_3 were too high. Kochankov *et al.* (1998) demonstrated that 60 mg/L GA_3 significantly promoted seed germination of *E. purpurea*, Our study verified that 200-800 mg/L GA_3 significantly promoted seed germination of *E. purpurea*, but not effective in higher concentrations at the range of 1000-2000 mg/L GA_3 (data not shown). Mcchia *et al.* (2001) found GA_3 (500mg/L) had no effect on the seed germination of *E. angustifolia*, while low concentration of GA_3 (0.1-0.3mg/L) conducive

to seed germination (Duan *et al.*, 2004). These results suggest seeds of different species show different sensitivities to GA_3.

No significant difference in germination percentage was observed between seeds treated at constant temperature and alternating temperature, this was consistent with ISTA rules for seed germination of *E. purpurea*. Similar results were obtained by Wartidiningsih and Geneve (1994). However, Macchia *et al.* (2001) found that seeds of *E. angustifolia* had a higher germination percentage at alternating temperature compare to at constant temperature. This might be due to the different species and cultural environments. Alternating temperature significantly shortened the MGT of seed of *E. purpurea* in our experiments, this result was consistent with AOSA rules for seed germination of *E. purpurea*, but different from ISTA rules.

Seed germination is promoted by GA_3 in many plant species. GA_3 is an important endogenous growth regulator that has profound and diverse effects on plant growth and development. Results of the present study support the proposal that GA_3 affects physiological and metabolic activities of seeds resulting in early germination (Duan *et al.*, 2004).

2. Ethephon enhanced germination percentage and shortened MGT

In our experiments, ethephon significantly promoted seed germination of *E. purpurea*. Several members of the Asteraceae family, including *E. purpurea* and *E. angustifolia*, require ethylene to break seed dormancy (Smith-Jochum and Albrecht, 1987; Kochkankov *et al.*, 1998; Abeles and Lonski, 1969; Corbineau and Côme, 1987; Katonh, 1975; Feghahati and Reese, 1994; Jones, 1968). Pill and Haynes (1996) reported the addition of ethephon during priming of *E. purpurea* seeds did not promote germination and attributed this to the lack of dormancy in their batch of *E. purpurea* seeds. It has been suggested that ethylene stimulates the synthesis or release of growth promoters necessary for germination (Ketring and Morgan, 1969). It has been shown that ethylene was synthesized in seeds, involved in radical elongation and it increased germination of after-ripened embryos (Jones, 1968; Olatoye and Hall, 1972).

3. Light and prechilling treatment promoted seed germination

According to International Rules for Seed Testing (ISTA, 2004), prechilling can be used for breaking dormancy. It has been used for various dormant seeds and has been reported to successfully alleviate endogenous dormancy. In our experiments, prechilling resulted in significant increases in percentage and rate of germination, both in the dark and in the light. There are conflicting results in the literature regarding the length of the of cold-moist treatment, with variation from 2 to 15 weeks (Baskin *et al.*, 1992; Parmenter *et al.*, 1996; Carlma *et al.*, 1993; Abeles and Lonski, 1969). In our experiments, we conclude that 14-day prechilling was optimal for maximum germination. Additional prechilling had insignificant effect.

In our study, seeds prechilled in the light had a higher germination percentage and germination rate compared to seeds prechilled in the dark. Light was considered a necessary factor in enhancing *E. angustifolia* seed germination (Macchia *et al.*, 2001) Parmenter *et al.*, 1996). In *E. purpurea*, the effect of light on seed germination was variable, either

promoting seed germination (Smith-Jochum and Albrecht, 1987) or having no effect (Wartidingsih and Geneve, 1994). The variance might be caused by different growing environments, because the environment of mother plants affects phototonus of seeds. In all cases light enhances seed germination of *E. purpurea* planted in South China.

4. Seed coat restricted seed respiratory rate

Mechanical scarification did not promote seed germination of *E. angustifolia* (Feghahati and Reese, 1994) and in our experiments seed germination of *E. purpurea* was not promoted when the pericarp was removed. But germination of *E. purpurea* was significantly increased if the seed coat was removed. Our results agreed with those reported by Duan *et al.* (2004), who found seed germination of *E. angustifolia* was promoted by removing the seed coat. It is not clear how seed coat restricted seed germination in *E. purpurea.* Commonly seed coat restricts seed germination in several ways, such as mechanical resistance, restricting seed water absorption, restricting seed respiration, the presence of germination inhibitor in the seed coat, or the seed coat restricting the leaching of inhibitor in the seed.

Our results suggested that the seed coat neither had mechanical resistance for seed germination (experiment 8) nor restricted water absorption (experiment 9). However, the results of experiment 10 showed that seed coat restricted seed respiration. Our observations clearly revealed removal of the seed coat enhanced germination by 96 hours compared to the control (figure 1). In addition seed germination percentage increased from 64.2% to 98.3% and MGT was shortened from 13.7 days to 4.5 days when the seed coat was removed (table 5). Because those phenomena were closely involved with the increase of seed respiration, we conclude that the dormancy of *E. purpurea* seeds is affected by seed coat restriction on the respiratory rate.

5. Endogenous ethylene produced by damaging seeds promoted seed germination

Our experiment revealed that treatment of cutting to equally divide the seeds promoted seed germination of *E. purpurea.* Endogenous ethylene is produced when seeds are damaged and it was proved that ethylene promoted seed germination of *E. purpurea* (Greenwood *et al.,* 1980; Kochakov *et al.,* 1998). We presume that damaged seeds synthesized endogenous ethylene which in turn improved seed germination of *E. purpurea.* To verify our hypothesis, enzyme inhibitor $Co(NO_3)_2$ was employed to inhibit ethylene synthesis. The result showed that germination of damaged seeds were prevented by ethylene inhibitor $Co(NO_3)_2$. Additionally experiment 10 further proved that damaged seeds indeed produced endogenesis ethylene. On the basis of the results, we conclude that endogenous ethylene promotes seed germination of *E. purpurea.*

Conclusion

Prechilling, light and use of plant hormones (GA_3 or ethylene) promoted seed germination of *E. purpurea*, but the highest germination rates were obtained when all the three treatments were used simultaneously. The seed coat appears to affect seed dormancy

by restricting the respiratory rate. In addition, endogenous ethylene can promote seed germination of *E. purpurea*. Our experiments show that treatments which increase the penetration of oxygen to the embryos or enhance the production of endogenous ethylene promote seed germination of *E. purpurea*.

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