

## ABA 对黑黄檀种子萌发的抑制作用以及其他植物激素对 ABA 的拮抗作用\*

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**摘要:** 以云南特有濒危树种黑黄檀 (*Dalbergia fusca*) 的种子为材料, 研究了脱落酸 (ABA) 对种子萌发的抑制作用, 以及种子萌发过程中吲哚乙酸 (IAA)、赤霉素 ( $GA_3$ )、6-苄基腺嘌呤 (6-BA) 和乙烯利对 ABA 的拮抗作用。黑黄檀种子萌发的适宜温度为 30 。交替光照 (14 h 光照和 10 h 黑暗) 以及黑暗对种子萌发没有明显的影响。0.001 ~ 0.1 mmol/L ABA 不影响种子的萌发率, 但降低种子的萌发进程; 1 mmol/L 和 2.5 mmol/L ABA 显著地抑制种子的萌发率和萌发进程。种子的萌发率不被 0.0001 ~ 1 mmol/L IAA 和  $GA_3$ 、0.0001 ~ 0.1 mmol/L 6-BA、以及 0.001 ~ 10 mmol/L 乙烯利 (乙烯供体) 的影响, 但被 1 mmol/L 6-BA 抑制。1 mmol/L ABA 对种子萌发的抑制作用能被 0.01 ~ 1 mmol/L IAA、0.01 ~ 1 mmol/L  $GA_3$ 、0.001 ~ 0.1 mmol/L 6-BA 和 0.1 ~ 10 mmol/L 乙烯利所拮抗, 而且这种拮抗作用与植物激素的类型和浓度有关。0.01 mmol/L 6-BA 和 0.1 mmol/L 乙烯利对 1 mmol/L ABA 抑制作用的拮抗不能被添加 0.001 mmol/L IAA 或者 0.001 mmol/L  $GA_3$  加成。但 0.1 mmol/L 乙烯利对 1 mmol/L ABA 抑制作用的拮抗能够被添加 0.01 mmol/L 6-BA 或者 0.1 mmol/L 6-BA 加成, 导致更高的萌发率和幼苗生长。

**关键词:** 脱落酸; 黑黄檀; 乙烯利; 植物激素的相互作用; 种子萌发

中图分类号: Q 945

文献标识码: A

文章编号: 0253-2700 (2008) 04-440-07

## Inhibitory Effect of ABA on Seed Germination of *Dalbergia fusca* (Leguminosae) and Antagonism of Other Phytohormones to ABA

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**Abstract:** Seeds of *Dalbergia fusca*, an endangered tree species endemic to Yunnan province of China were used to study the inhibitory effects of abscisic acid (ABA) and the antagonism of IAA,  $GA_3$ , 6-BA and ethephon to ABA in the seed germination. The optimum temperature for the seed germination was about 30. There was no different effects of alternating photoperiod (14 h light and 10 h dark) and darkness on the seed germination. After treated by 0.01-0.1 mmol/L ABA, the seed germination percentage was not affected, but the time course of germination was decreased, while those levels were dramatically inhibited by 1 mmol/L and 2.5 mmol/L ABA. The seed germination percentage was not affected by 0.0001 - 1 mmol/L indole-3-acetic acid (IAA), gibberellic acid ( $GA_3$ ), 0.0001 - 0.1 mmol/L 6-benzyladenine (6-BA), and 0.001 - 10 mmol/L ethephon (the ethylene donor), but was inhibited by 1 mmol/L 6-BA. The inhibition effect of 1 mmol/L ABA on seed germination was antagonized by 0.01-1 mmol/L IAA, 0.01-1 mmol/L  $GA_3$ , 0.001 - 0.1 mmol/L 6-BA and 0.1

\* Foundation item: Knowledge Innovation Program of the Chinese Academy of Sciences (KSCX2-SW-117, KSCX2-YW-Z-058)

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Received date: 2007-10-10, Accepted date: 2008-04-21

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-10 mmol/L ethephon, which were phytohormone type- and concentration-dependent. The antagonistic actions of 0.01 mmol/L 6-BA and 0.1 mmol/L ethephon to 1 mmol/L ABA inhibition could not be increased by addition of 0.001 mmol/L IAA or 0.001 mmol/L GA<sub>3</sub>. The antagonistic action of 0.1 mmol/L ethephon to 1 mmol/L ABA inhibition, however, could be increased by addition of 0.01 mmol/L 6-BA or 0.1 mmol/L 6-BA, which resulting in higher germination percentage and enhancing seedling growth.

**Key words:** Abscisic acid; *Dalbergia fusca*; Ethephon; Phytohormone interaction; Seed germination

The process of germination begins with the uptake of water by the dry seed, followed by the expansive embryo growth. This usually culminates in rupture of the covering layers and the emergence of the radicle, which is generally considered to be the completion of the germination. It has been reported that phytohormones regulate germination and dormancy of seeds. GA<sub>3</sub>, at high concentration, can break the dormancy of positive photoblastic seeds such as *Lactuca scariola*, negative photoblastic seeds such as *Phacelia tanacetifolia*, and non-photoblastic seeds which require stratification, such as *Avena fatua* (Berrie, 1984). Kinetin can induce germination in photosensitive lettuce seeds, and like GA, but a high concentration is needed (Berrie, 1984).

ABA is known as a positive regulator of seed dormancy and a negative regulator of seed germination (Hilhorst and Downie, 1995; Bewley, 1997; Koenneef *et al.*, 2002). ABA treatment of non-endospermic, non-dormant *Brassica napus* seeds has no effect on the kinetics of testa rupture, but inhibits the post-germination extension growth of the radicle (Schopfer and Plachy, 1985). ABA does not inhibit the initial imbibition of water (water uptake phase 1 and 2) needed for the initial extension growth of the embryo, but inhibits the transition to the seedling growth phase (water uptake phase 3) after radicle emergence (Lopez-Molina *et al.*, 2001).

In many species, ethylene stimulates germination of seeds that may or may not be dormant. Ethylene is effective in breaking the primary dormancy imposed by seed coat in cocklebur, subterranean clover and *Rumex crispus* seeds, and the embryo dormancy of apple and sunflower seeds. It can also overcome the thermo-dormancy in lettuce seeds and the secondary dormancy in cocklebur seeds (Corbineau and Côme, 1995).

*Dalbergia fusca* Pierre ex Prain (family Fabaceae) is a valuable timber tree endemic to Yunnan Province of

China. It is considered to be an endangered species and has been given protection by the Chinese government (State Environmental Protection Administration of China and Institute of Botany of Chinese Academy of Sciences, 1987). To our knowledge, the factors affecting the germination of *D. fusca* seed, including temperature, light and phytohormones, have not been reported. In this study, the inhibitory effects of ABA on the germination of *D. fusca* seed and the antagonism of IAA, GA<sub>3</sub>, 6-BA and ethephon to ABA were investigated.

## Materials and methods

**Plant material** Fruits of *Dalbergia fusca* were collected at maturity in December, 2004 from trees growing in Xishuangbanna Tropical Botanical Garden (101°25' E, 21°41' N; altitude 600 - 700 m), Menglun, Mengla, Yunnan, China. After removal from fruits, the seeds were dried for 14 days at 15 ± 1 °C in 50% relative humidity (RH) to a moisture content of 0.092 ± 0.004 g H<sub>2</sub>O g<sup>-1</sup> dry weight (DW) and then kept at 15 °C before the different experimental treatments.

**Moisture content determinations** The moisture content of the seeds is expressed on a dry weight basis (g H<sub>2</sub>O g<sup>-1</sup> DW, g g<sup>-1</sup>) and was determined by weighing 5 replicates of 100 seeds each after the seeds were dried for 48 h at 80 °C.

**Germination testing** Four replicates of 50 seeds each were germinated on two pieces of filter paper moistened with 7 ml of distilled water or of a water solution of phytohormone of indicated concentration in closed 9-cm diameter Petri dishes subjecting to different light and temperature regimes. Seeds showing 2-mm radicle emergence were counted as germinated. The germination rate is expressed on a time course of germination and/or the number of days taken to reach the 50% germination stage (T<sub>50</sub>).

**Statistical analysis** The effects of temperature, light and phytohormones on seed germination were analyzed using a one-way ANOVA model from the SPSS 11.5 package for Windows (SPSS Inc.).

## Results

### Effects of temperature and light on seed germination

The weight of 1000 seeds of *D. fusca* was  $46.05 \pm 0.73$  g. When the seeds were germinated in darkness at 15, 20, 25, 30, 35 and 40 °C respectively, the germination percentage and germination rate of seeds were markedly affected by temperature, the time required for 50% germination of seeds ( $T_{50}$ ) was about 80 h at 15 °C, 40 h at 20 °C, 29 h at 25 °C, 25 h at 30 °C, 27 h at 35 °C and 62 h at 40 °C (Fig. 1: a). The optimum temperature for final germination percentage and  $T_{50}$  was about 30 °C, and for seedling growth, about 35 °C (Fig. 1: b).

The alternating photoperiod [14 h light ( $12 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and 10 h dark] and darkness had no effect on the final germination percentage and  $T_{50}$  at 30 °C (Fig. 1: c). The fresh weight of seedlings produced by germinating seeds in the alternating photoperiod was a little lower than in darkness (Fig. 1: d).

#### Inhibitory effects of abscisic acid on seed germination

0.001 - 0.1 mmol/L ABA did not affect the seed germination percentage, but decreased the time course of germination; 1 and 2.5 mmol/L ABA dramatically inhibited seed germination and decreased the time course of germination (Fig. 2: a). Except that 0.0001 mmol/L ABA slightly stimulated seedling growth (data not shown), 0.001 - 1 mmol/L ABA inhibited seedling growth (Fig. 2: b).

#### Effects of IAA, GA<sub>3</sub>, 6-BA and ethephon on seed germination

At 30 °C, the germination percentage of seeds was not affected by 0.0001 - 1 mmol/L IAA, 0.0001 - 1 mmol/L GA<sub>3</sub>, 0.0001 - 0.1 mmol/L 6-BA, and 0.001 - 10 mmol/L ethephon (the ethylene donor), but was inhibited by 1 mmol/L 6-BA (Table 1).

The fresh weight of the seedlings produced by germinating seeds was increased by 0.0001 mmol/L and 0.001 mmol/L IAA, 0.0001 - 0.1 mmol/L GA<sub>3</sub>, 0.0001

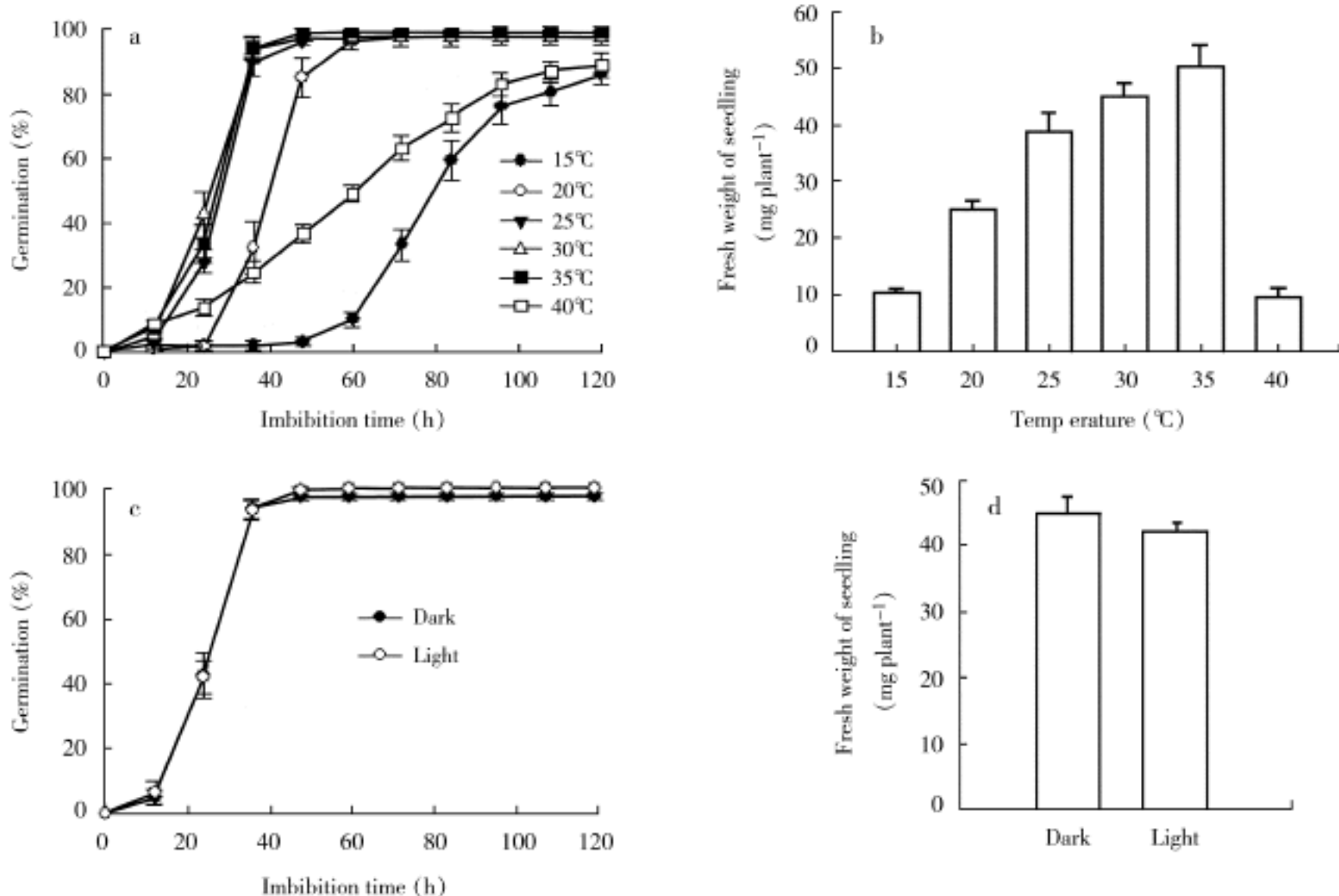


Fig. 1 Effects of temperature and light on germination percentage and rate of *D. fusca* seeds

a. seeds were germinated at 15, 20, 25, 30, 35 and 40 °C, respectively, and in darkness for 5 d; b. Fresh weight of seedlings produced by germinating seeds (excluding cotyledons); c. seeds were germinated at 30 °C and alternating photoperiod (14 h light/10 h dark) or darkness for 5 d; d. Fresh weight of seedlings produced by germinating seeds (excluding cotyledons) at 30 °C with alternating photoperiod or darkness. Seeds showing radicle emergence for 2 mm were scored as germinated. All values are means  $\pm$  SD of four replicates of 50 seeds each.

Table 1 Effects of phytohormones on germination of *Dalbergia fusca* seeds .Seeds were germinated at indicated concentrations of phytohormones for 5 d (dark, 30 °C) . Seeds showing radicle emergence for 2 mm were scored as germinated . All values are means  $\pm$  SD of four replicates of 50 seeds each

Concentration (mmol/L)	Phytohormone			
	IAA	GA	6-BA	Ethephon
0	100.0 $\pm$ 0.0	99.5 $\pm$ 0.5	100.0 $\pm$ 0.0	99.5 $\pm$ 0.5
0.0001	99.5 $\pm$ 0.5	100.0 $\pm$ 0.0	99.0 $\pm$ 1.0	—
0.001	99.0 $\pm$ 1.0	99.5 $\pm$ 0.5	99.0 $\pm$ 0.5	100.0 $\pm$ 0.0
0.01	98.5 $\pm$ 1.0	100.0 $\pm$ 0.0	99.0 $\pm$ 0.5	100.0 $\pm$ 0.0
0.1	98.5 $\pm$ 1.0	99.0 $\pm$ 1.0	99.0 $\pm$ 0.5	100.0 $\pm$ 0.0
1	99 $\pm$ 1.0	99.5 $\pm$ 0.5	71.3 $\pm$ 6.4	100.0 $\pm$ 0.0
10	—	—	—	100.0 $\pm$ 0.0

Table 2 Effects of phytohormones on fresh weight (mg plant) of seedlings produced by germinating *Dalbergia fusca* seeds . Seeds were germinated at indicated concentrations of phytohormones for 5 d (dark, 30 °C) . Fresh weight of seedling does not include cotyledons . All values are means  $\pm$  SD of four replicates of 50 seeds each

Concentration (mmol/L)	Phytohormone			
	IAA	GA	6-BA	Ethephon
0	42.78 $\pm$ 4.20	43.95 $\pm$ 3.01	42.93 $\pm$ 2.12	44.62 $\pm$ 1.03
0.0001	44.83 $\pm$ 2.13	44.21 $\pm$ 6.01	43.13 $\pm$ 2.77	44.10 $\pm$ 3.16
0.001	45.16 $\pm$ 3.33	48.05 $\pm$ 3.18	46.00 $\pm$ 3.15	45.85 $\pm$ 3.20
0.01	42.53 $\pm$ 4.72	53.88 $\pm$ 4.09	48.73 $\pm$ 1.41	51.16 $\pm$ 3.36
0.1	39.55 $\pm$ 5.26	51.21 $\pm$ 3.51	40.33 $\pm$ 1.45	44.74 $\pm$ 1.93
1	26.73 $\pm$ 2.88	43.74 $\pm$ 4.13	4.84 $\pm$ 0.13	42.70 $\pm$ 1.08
10	—	—	—	26.30 $\pm$ 0.79

- 0.01 mmol/L 6-BA, and 0.001 mmol/L and 0.01 mmol/L ethephon, but was inhibited by 0.1 mmol/L and 1 mmol/L IAA, 1 mmol/L GA<sub>3</sub>, 0.1 mmol/L and 1 mmol/L 6-BA, and 1 mmol/L and 10 mmol/L ethephon (Table 2) .

#### Antagonism of IAA, GA<sub>3</sub>, 6-BA and ethephon to ABA during seed germination

The germination percentage, the time course of germination and the seedling growth were significantly inhibited by 1 mmol/L ABA (Fig. 2 and 3a), which could be antagonized by 0.01-1 mmol/L IAA, 0.01-1 mmol/L GA<sub>3</sub> (Fig. 3: a), 0.001-0.1 mmol/L 6-BA and 0.1 - 10 mmol/L ethephon (Fig. 3: b). In these antagonisms to ABA, the effect of ethephon was the largest, GA<sub>3</sub> and 6-BA, the medium, and IAA, the smallest . The optimum antagonistic concentration to 1 mmol/L ABA was

0.01 mmol/L for IAA and GA<sub>3</sub>, 0.1 mmol/L for 6-BA, and 10 mmol/L for ethephon (Fig. 3: a and b) .

It was noted that 0.01 - 0.1 mmol/L IAA, 0.01 - 0.1 mmol/L GA<sub>3</sub>, and 0.001 - 0.1 mmol/L 6-BA increased, and 0.01 - 1 mmol/L ethephon markedly increased, the fresh weight of seedlings in antagonism to ABA (data not shown) .

#### Interaction among phytohormones during seed germination

ABA inhibition for seed germination (Fig. 4), the time course of germination and seedling growth (data not shown) was notably antagonized by 0.01 mmol/L 6-BA and 0.1 mmol/L ethephon, which could not be increased by addition of 0.001 mmol/L IAA or GA<sub>3</sub>, but could be increased by addition of 6-BA (0.01 or 0.1 mmol/L) or ethephon (0.1 mmol/L) (Fig. 4) .

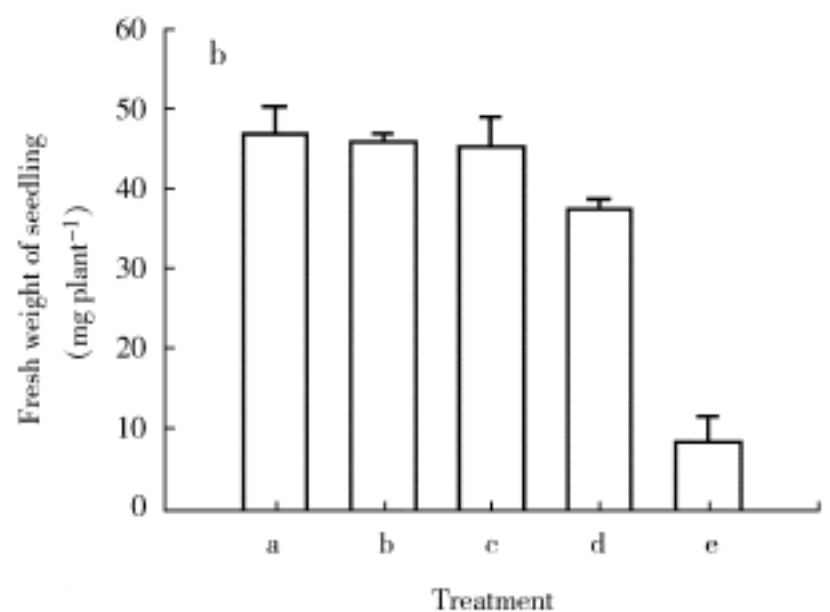
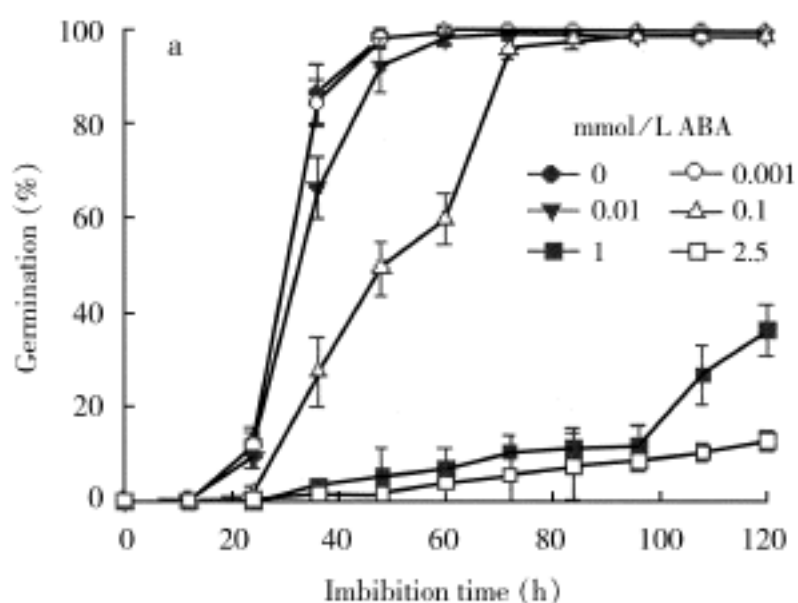


Fig. 2 Effects of ABA on germination percentage (a) and fresh weight of seedling (b) of *D. fusca* seeds . Seeds were germinated at indicated concentrations of ABA for 5 d (dark, 30 °C) . Seeds showing radicle emergence for 2 mm were scored as germinated .

Fresh weight of seedling does not include cotyledons . a, 0 mmol/L ABA; b, 0.001 mmol/L ABA; c, 0.01 mmol/L ABA; d, 0.1 mmol/L ABA; e, 1 mmol/L ABA; All values are means  $\pm$  SD of four replicates of 50 seeds each

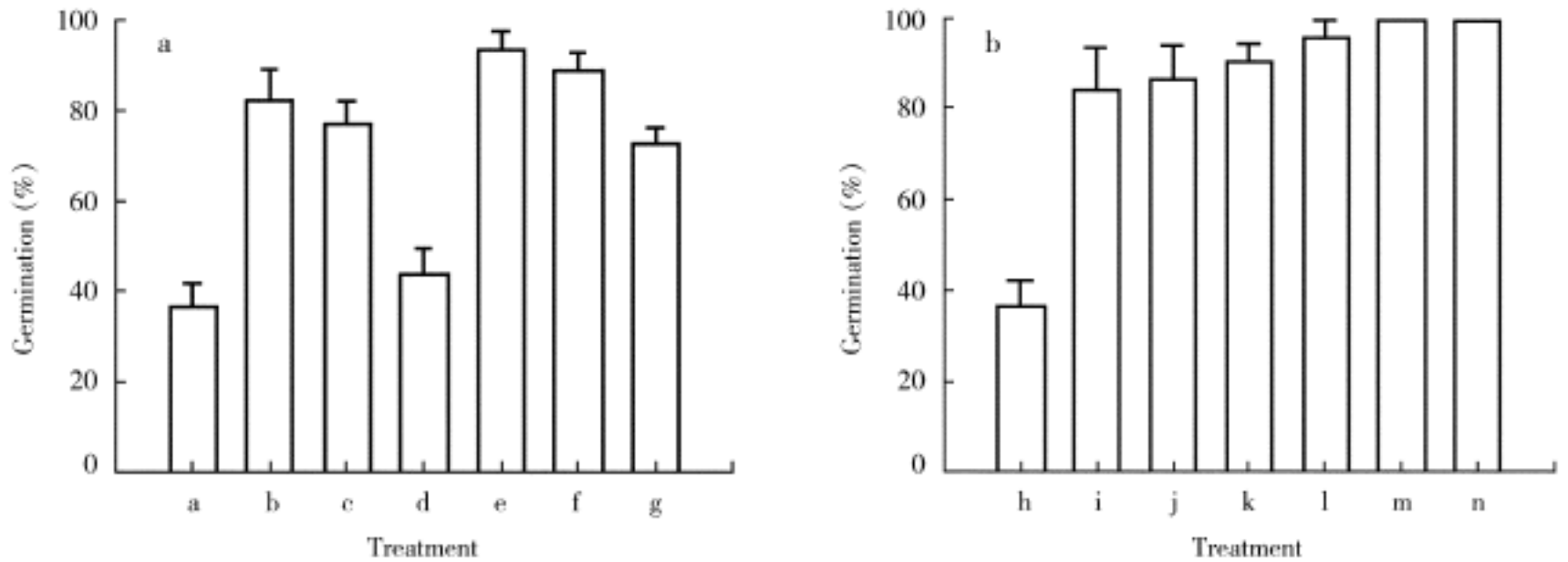


Fig. 3 Antagonism of IAA, GA<sub>3</sub>, 6-BA and ethephon to ABA. Seeds of *D. fusca* were germinated at different treatments, respectively, for 5 d (dark, 30 °C). Seeds showing radicle emergence for 2 mm were scored as germinated. a and h, 1 mmol/L ABA; b, 1 mmol/L ABA + 0.01 mmol/L IAA; c, 1 mmol/L ABA + 0.1 mmol/L IAA; d, 1 mmol/L ABA + 1 mmol/L IAA; e, 1 mmol/L ABA + 0.01 mmol/L GA<sub>3</sub>; f, 1 mmol/L ABA + 0.1 mmol/L GA<sub>3</sub>; g, 1 mmol/L ABA + 1 mmol/L GA<sub>3</sub>; i, 1 mmol/L ABA + 0.001 mmol/L 6-BA; j, 1 mmol/L ABA + 0.01 mmol/L 6-BA; k, 1 mmol/L ABA + 0.1 mmol/L 6-BA; l, 1 mmol/L ABA + 0.1 mmol/L ethephon; m, 1 mmol/L ABA + 1 mmol/L ethephon; n, 1 mmol/L ABA + 10 mmol/L ethephon. All values are means ± SD of four replicates of 50 seeds each

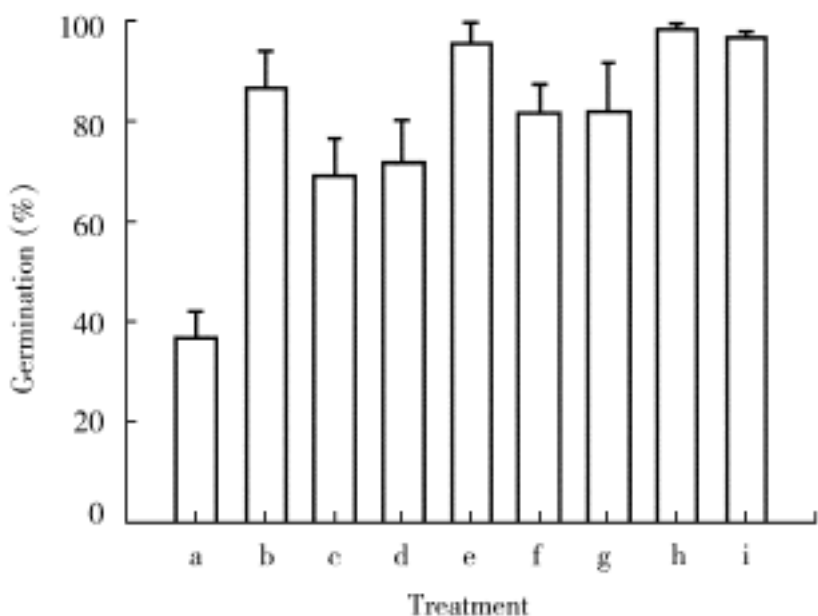


Fig. 4 Interaction among phytohormones during germination of *D. fusca* seeds. Seeds were germinated at different treatments, respectively, for 5 d (dark, 30 °C). Seeds showing radicle emergence for 2 mm were scored as germinated. a, 1 mmol/L ABA; b, 1 mmol/L ABA + 0.01 mmol/L 6-BA; c, 1 mmol/L ABA + 0.01 mmol/L 6-BA + 0.001 mmol/L IAA; d, 1 mmol/L ABA + 0.01 mmol/L 6-BA + 0.001 mmol/L GA<sub>3</sub>; e, 1 mmol/L ABA + 0.1 mmol/L ethephon; f, 1 mmol/L ABA + 0.1 mmol/L ethephon + 0.001 mmol/L IAA; g, 1 mmol/L ABA + 0.1 mmol/L ethephon + 0.001 mmol/L GA<sub>3</sub>; h, 1 mmol/L ABA + 0.1 mmol/L ethephon + 0.01 mmol/L 6-BA; i, 1 mmol/L ABA + 0.1 mmol/L ethephon + 0.1 mmol/L 6-BA. All values are means ± SD of four replicates of 50 seeds each

## Discussion

Among the several environmental factors which affect germination, temperature is the single most important factor governing both the maximum germination

percentage and the germination rate (Heydecker, 1977; Huang *et al.*, 2003). Temperature affects both the capacity for germination and the rate of germination (Bewley and Black, 1994).  $T_{50}$  for *D. fusca* seed was about 80 h at 15 °C, 40 h at 20 °C, 29 h at 25 °C, 25 h at 30 °C, 27 h at 35 °C and 62 h at 40 °C (Fig. 1: a). The optimum temperature for germination percentage and  $T_{50}$  was about 30 °C, and for seedling growth, about 35 °C (Fig. 1: b). These results are in agreement with the viewpoint of Heydecker (1977) and Huang *et al.* (2003).

The alternating photoperiod (14 h light and 10 h dark) or darkness had no effect on germination percentage and  $T_{50}$  at 30 °C (Fig. 1: c), and had a little effect on the fresh weight of the seedlings produced by germinating seeds (Fig. 1: d), showing that the seed of *D. fusca* was non-photoblastic.

0.001 - 0.1 mmol/L ABA did not affect the germination percentage of the seeds, but it affected the time course of the germination and the seedling growth (Fig. 2). 1 and 2.5 mmol/L ABA dramatically inhibited the germination percentage, the time course of germination and the subsequent seedling growth (Fig. 2). It has been reported that the addition of exogenous ABA to the medium during imbibition resembles the effects of maternal ABA during seed development and residual

ABA in mature seeds. It has been also reported that the imbibition of fresh or after-ripened tobacco seeds in medium with 10  $\mu\text{mol/L}$  ABA greatly delays seed germination (Leubner-Metzger, 2003).

The germination percentage of the seeds was not affected by 0.0001-1 mmol/L IAA, 0.0001 - 1 mmol/L  $\text{GA}_3$ , 0.0001 - 0.1 mmol/L 6-BA, and 0.001 - 10 mmol/L ethephon (Table 1), implying that *D. fusca* seeds might have sufficient endogenous IAA, GAs, cytokinins and ethylene required for germination. The germination percentage of the seeds was inhibited by 1 mmol/L 6-BA (Table 1). The seedling growth after germination was enhanced by 0.0001 mmol/L and 0.001 mmol/L IAA, 0.0001 - 0.1 mmol/L  $\text{GA}_3$ , 0.0001 - 0.01 mmol/L 6-BA, and 0.001 mmol/L and 0.01 mmol/L ethephon, but was inhibited by 0.1 mmol/L and 1 mmol/L IAA, 1 mmol/L  $\text{GA}_3$ , 0.1 mmol/L and 1 mmol/L 6-BA, and 1 mmol/L and 10 mmol/L ethephon (Table 2). These results also indicated that the effects of IAA,  $\text{GA}_3$  and 6-BA on the seed germination and subsequent growth were phytohormone type- and concentration-dependent. Seed germination of the GA-deficient biosynthesis mutant *gal* of *Arabidopsis* depends on the addition of GA to the medium during imbibition (Koornneef and Karssen, 1994). Studies with the GA-deficient *gib-1* clearly showed that GAs control the endosperm breakdown. The mutant seed only germinated in the presence of GA or after removal of the covering layers opposing the radicle (detipping) (Groot *et al.*, 1987). The germination of detipped *gib-1* seeds in water indicated a location of GA action in the covering layers surrounding the tip of the radicle. Measurements of the puncture force required to break through these layers showed that the major action of endogenous GA was directed to the weakening of the mechanical resistance of the endosperm cells around the radicle tip (Groot *et al.*, 1987). In wild-type seeds the endosperm weakening occurred in water before radicle protrusion; in *gib-1* seeds it was absolutely dependent on applied  $\text{GA}_{4+7}$ . Simultaneously incubation of deembryonated endosperm and isolated axes showed that only wild-type embryos produce a factor that induces endosperm weakening, that probably is GA. GA can induce  $\alpha$ -amylase synthesis, and stimulate mobilization of stored

reserves (Bewley and Black, 1994).

The fresh weight of the seedlings produced by germinating seeds was enhanced by 0.0001 - 0.01 mmol/L 6-BA (Table 2), showing that 6-BA could stimulate growth in an unknown manner. Cytokinins are very effective promoters of germination, since early protein synthesis is a necessary event for seed germination, and exogenously applied cytokinin could enhance protein synthesis (Berrie, 1984).

The germination percentage, the time course of germination and the seedling growth were significantly inhibited by 1 mmol/L ABA (Fig. 2 and 3a), which were markedly antagonized by 0.01 - 1 mmol/L IAA, 0.01 - 1 mmol/L (Fig. 3: a), 0.001 - 0.1 mmol/L 6-BA and 0.1 - 10 mmol/L ethephon (Fig. 3: b). However, the antagonistic effects of IAA,  $\text{GA}_3$ , 6-BA and ethephon to ABA were unknown. In these antagonisms to ABA, the effect of ethephon was the largest,  $\text{GA}_3$  and 6-BA, the medium, and IAA, the smallest (Fig. 3), showing that these antagonisms were also phytohormone type- and concentration-dependent. Ethylene is also known to allow dormant sunflower embryos to germinate in hypoxia (Corbineau and Côme, 1992), overcomes the inhibition of germination imposed by osmotic agents in *Amaranthus caudatus* seeds (Kepczynski and Karssen, 1985; Kepczynski, 1986).  $\alpha$ -1, 3-glucanase is implicated in the after-ripening-mediated promotion of tobacco testa and endosperm rupture. Class I  $\alpha$ -1, 3-glucanase (Glu I) is transcriptionally induced in germinating tobacco seeds just prior to endosperm rupture but after testa rupture (Leubner-Metzger *et al.*, 1995, 1998). Glu I induction is highly localized in the micropylar endosperm at the site of radicle emergence. Light, GA and ethylene promote Glu I expression and endosperm rupture. ABA inhibits Glu I expression and endosperm rupture of wild-type seeds (Leubner-Metzger, 2003). The slow growth rate of the radicles that emerge from detipped *gib-1* seeds indicates GAs also control embryo growth as part of germination. Probably embryo growth is primarily controlled at the extensibility of the cell walls (Schopfer and Plachy, 1985). The inhibitory action of applied ABA on germination has indeed been related to reducing

cell-wall extensibility (Karssen, 1995) .

It has been shown that 0.01 mmol/L 6-BA + 0.001 mmol/L IAA or + 0.001 mmol/L GA<sub>3</sub> , and 0.1 mmol/L ethephon + 0.001 mmol/L IAA or + 0.001 mmol/L GA<sub>3</sub> for antagonism to 1 mmol/L ABA had no synergic role (Fig . 4) , but that 0.1 mmol/L ethephon + 0.01 mmol/L 6-BA or + 0.1 mmol/L 6-BA had a synergic role on antagonism to 1 mmol/L ABA, significantly increasing the germination percentage (Fig . 4) and the seedling growth (data not shown) .

To our knowledge, It was first reported that inhibition by ABA for seed germination could be antagonized by IAA, GA<sub>3</sub> , 6-BA, ethephon, 6-BA + IAA or + GA<sub>3</sub> , ethephon + IAA or + GA<sub>3</sub> , and ethephon + 6-BA; and that 6-BA + IAA or + GA<sub>3</sub> , and ethephon + IAA or + GA<sub>3</sub> had no synergic role, and that ethephon + 6-BA had a synergic role, for antagonism to ABA . These roles were also phytohormone type- and concentration-dependent . However, these antagonism mechanisms are unknown, and deserve further research . It might be considered that effects of phytohormones on seed germination might become a model system for studying interactions among phytohormones .

**Acknowledgements:** The authors are grateful to Professor Jin Xiao-bai (Institute of Botany, Chinese Academy of Sciences) for revising the paper .

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