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脱落酸引发对夜香紫罗兰种子萌发的效应

张志胜[1]，DORNA Hanna[2]
（1. 华南农业大学农学院，广州 510642；2. 波兹南农业大学种子科学技术系，波兹南 Baranovo，62-081，波兰）

摘要：研究脱落酸引发及其与植物生长调节剂的复合处理对夜香紫罗兰(*Matthiola bicornis*)种子萌发的效应。结果表明，将夜香紫罗兰种子于 20℃下在 2.5×10^{-5} mol/L 脱落酸(ABA)溶液中引发 7 d，能显著缩短种子平均萌发时间，增加种子萌发指数。在 ABA 溶液中加入吲哚乙酸(NAA)未能进一步改善夜香紫罗兰种子萌发，但其他复合处理均显著提高正常幼苗百分率。ABA 和细胞分裂素(KT)及 NAA 复合处理进一步增加种子萌发指数，不仅比单独用 ABA 引发的效果好，而且也比用聚乙二醇(PEG)引发的效果好。ABA 引发和 PEG 引发的夜香紫罗兰种子相对吸水量显著不同。对 ABA 引发机理和应用潜力进行了讨论。

关键词：夜香紫罗兰；脱落酸；种子引发；种子萌发
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The Effects of ABA Priming on Germination of *Matthiola bicornis* Seeds

ZHANG Zhi-Sheng[1]，DORNA Hanna[2]
（1. *College of Agriculture, South China Agricultural University*, Guangzhou 510642, China；
2. *Dept of Seed Science and Technology, Poznán Agricultural University*, Baranovo, 62-081 Przezmierovo, Poland）

Abstract: The effects of ABA priming, both alone and in combination with GA_3, KT and NAA on germination of *Matthiola bicornis* seeds were investigated. The best priming was to treat seeds in 2.5×10^{-5} mol/L ABA solution at 20℃ for 7 d. This treatment significantly reduced mean germination time and increased germination index compared with the control. Combined treatment by ABA with NAA showed no further effect on germination of the seeds, but all the other combined treatments significantly increased the percentage of normal seedlings. The treatment in combination with KT and NAA increased the germination index compared to the treatment with ABA or PEG priming alone. There were quite differences in the seed relative water absorption between treatments with PEG and ABA priming. The potential and the mechanisms for using ABA priming to improve seed germination are discussed.

Key words: *Matthiola bicornis*; ABA; Seed priming; Seed germination

Seed treatment can improve seed quality and becomes more and more important in modern seed industry[1]. Seed priming first coined by Heydecher et al.[2] for the controlled hydration of seeds is an important and effective seed treatment method to achieve germination advancing and synchronizing[3-6], and several seed treatment methods have been developed[1,7]. Exogenous ABA can reversibly block the process of germination[8], and ABA treatment significantly increased the synchrony of carrot seed germination[9]. Pre-treating tomato seeds in ABA solution significantly enhanced seedling emergence and gave very similar results to that of osmotic priming[10]. ABA seed pre-treatment significantly increased the plant growth and K^+ concentration in the plant of salinity sensitive wheat variety[11]. However, Carneiro et al[12] reported that ABA treatment delayed seed germination of alfalfa, seeds maintained in ABA had very low

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germination, and the interaction of ABA+GA_3 was not sufficient to overcome the action of the inhibitor. Up to now, there were few reports in literature on the response of seeds to ABA priming (treated seeds as osmotic priming). The main goal of the present study was to investigate the potential for using ABA priming, both alone and in combination with other plant growth regulators, to improve germination of *Matthiola bicornis* seeds.

1 Materials and Methods

1.1 Plant material

Seeds of *Matthiola bicornis* DC used as material were kindly granted by Poznán Horticultural Seed Company "CNOS". The chemicals of abscisic acid (ABA), gibberellin$_3$ (GA_3), kinetin (KT), naphthylacetic acid (NAA) and polyethylene glycol (PEG) were purchased from Sigma Company.

1.2 ABA priming

For each treatment, six-replicates of fifty seeds were placed in Petri dishes lined with four layers of filter paper moistened with 5 ml of ABA solution at 2.5×10^{-5}, 5.0×10^{-5}, 7.5×10^{-5} or 1.0×10^{-4} mol/L concentration. Petri dishes were sealed with parafilm and placed in culture room at 20℃ for 7 d. Following treatment, seeds were washed under running tap water for 1 min and next rinsed three times in distilled water. Then all seeds were dried back at 20℃ and 45% ± 5% relative humidity for 48 h. After drying, seeds were set up for germination. The untreated seeds were the control.

1.3 PEG priming

Preliminary experiments showed that the best priming conditions for *Matthiola bicornis* seeds were to prime seeds in −1.25 MPa PEG solution at 15℃ for 7 d. Seeds were primed in the same way as ABA priming except for using PEG solution instead of ABA.

1.4 Combined treatment

The most effective concentration of ABA 2.5×10^{-5} mol/L for improvement germination of *M. bicornis* seeds was selected from the germination test and was used in combination with 1.4×10^{-4} mol/L GA_3, 7.0×10^{-5} mol/L KT, 4.7×10^{-5} mol/L KT, 2.9×10^{-5} mol/L GA_3 + 4.7×10^{-5} mol/L KT, 2.9×10^{-5} mol/L GA_3, 5.4×10^{-5} mol/L NAA, 2.7×10^{-5} mol/L NAA, 2.9×10^{-5} mol/L GA_3 + 5.4×10^{-5} mol/L NAA, 4.7×10^{-5} mol/L KT + 1.4×10^{-5} mol/L NAA, 2.9×10^{-5} mol/L GA_3 + 4.7×10^{-5} mol/L KT + 5.4×10^{-5} mol/L NAA, respectively. Seeds were treated in the same way as described above. After treatment, seeds were set up for germination.

1.5 Seed germination test

In each germination test, six replicates of 50 seeds were placed in 9 cm diameter Petri dishes lined with four layers of filter paper moistened with distilled water, and then incubated at 20℃ in darkness. The number of seeds germinated (the length of radical is no shorter than the length of the seed) was recorded daily until no further germination occurred. The final count and evaluation of normal seedlings and ungerminated fresh seeds took place after 14 d according to International Seed Testing Association rules [13]. Mean germination time, germination index were calculated according to the following formula:

$$\text{Mean germination time} = \frac{d_1\times a_1+d_2\times a_2+\cdots+d_i\times a_i}{a_1+a_2+\cdots+a_i}$$

$$\text{Germination index} = \frac{a_1}{d_1}+\frac{a_2}{d_2}+\cdots+\frac{a_n}{d_n}$$

Where $d_i = d_1, d_2, \cdots d_n$, the number of days of germination; $a_i = a_1, a_2, \cdots a_n$, the number of germinated seeds during the first day, the second day.....etc.

The percentage of normal seedlings and ungerminated fresh seeds were also calculated.

1.6 Relative water absorption determination

Two sets of *Matthiola bicornis* seeds (each set comprised of six replicates of 50 seeds) (W_1) were treated in ABA solution containing various plant growth regulators or in PEG solution as mentioned above. Following treatment, seeds of each set were harvested together and washed under running tap water for 2 min next rinsed in distilled water three times. Then, the seeds were surface dried with filter paper and weighed on an analysis balance (W_2). The relative water absorption was calculated as follows:

Relative water absorption (%)= $(W_2 - W_1)/W_1 \times 100$

The control was the relative water absorption of seeds which just began to germinate on filter paper moistened with distilled water.

2 Results

2.1 Germination response of seeds to ABA priming

Germination response of *Matthiola bicornis* seeds to ABA priming depended on ABA concentration. Seeds treated with 1.25×10^{-5} mol/L ABA germinated during the priming. Priming seeds in 2.5×10^{-5} mol/L ABA solution at 20℃ for 7 d significantly increased germination index and reduced mean germination time compared to untreated seeds (Table 1). Although treating seeds in 5.0×10^{-5} mol/L and 7.5×10^{-5} mol/L ABA solution reduced mean germination time and increased germination index, the differences were not significant at statistical level. Seeds treated in 1.0×10^{-4} mol/L ABA solution showed slower germination and thepercentage of normal seedlings was significantly decreased compared to the control. Hence, the concentration of 2.5×10^{-5} mol/L was best for ABA priming of *Matthiola bicornis* seeds and was selected for further experiment.

It should be noted that all ABA treatments significantly increased percentage of ungerminated fresh seeds, which indicated that ABA priming could increase dormancy of the seeds.

2.2 The effects of ABA priming, both alone and in combination with GA_3, KT and NAA, on germination of the seeds

Compared with the control, priming *Matthiola bicornis* seeds in 2.5×10^{-5} mol/L ABA solution significantly increased germination index and reduced mean germination time, but did not affect the final percentage of normal seedlings. These results were similar to that of PEG priming (Table 2). There were fewer bacteria and fungus diseases to occur during ABA priming, however, it was more severe during PEG priming.

Priming seeds in 2.5×10^{-5} mol/L ABA + 2.9×10^{-4} mol/L GA_3, 2.5×10^{-5} mol/L ABA + 7.0×10^{-5} mol/L KT, 2.5×10^{-5} mol/L ABA + 4.7×10^{-5} mol/L KT and 2.5×10^{-5} mol/L ABA + 2.9×10^{-5} mol/L GA_3 + 4.7×10^{-5} mol/L KT solution at 20℃ for 7 d, respectively, resulted in seed germination during the treatments. The percentages of germinated seeds were 35.0%, 87.0%, 85.3% and 85.0%, respectively. This showed that both KT and high concentration GA_3 could relieve the action of ABA to block seed germination. Seeds treated in 2.5×10^{-5} mol/L ABA + 4.7×10^{-5} and 7.0×10^{-5} mol/L KT solution germinated more uniformly than those in 2.5×10^{-5} mol/L ABA + 2.9×10^{-4} mol/L GA_3 solution. It suggested that KT was more effective than GA_3.

All combined treatments (Table 2) significantly reduced mean germination time and increased

Table 1 Germination response of *Matthiola bicornis* seeds to various concentrations of ABA priming at 20℃

ABA (mol/L)	Mean germination time (d)	Germination index	Normal seedlings (%)	Ungerminated fresh seeds (%)
0	1.99 b	45.77 b	77.7 a	7.0 c
2.5×10^{-5}	1.32 c	58.03 a	69.0 a	19.7 a b
5.0×10^{-5}	1.72 b	52.00 a	74.0 a	13.7 b
7.5×10^{-5}	1.80 b	46.53 b	70.3 a	19.7 a b
1.0×10^{-4}	2.50 a	28.58 c	56.0 b	31.3 a

Means in the same column followed by the same letter are not significantly different at $\alpha = 0.05$ by Duncan's multiple range test. The maximum value of germination index is 100, and the minimum 0.

Table 2 The effects of ABA priming alone and in combination with GA_3, KT, NAA on germination of *Matthiola bicornis* seeds

Treatment	Mean germination time (d)	Germination index	Normal seedlings (%)	Ungerminated fresh seeds (%)
Control	1.99 a	45.77 e	77.7 bcd	6.7 bc
I	1.34 d	71.77 b	82.0 abc	0.7 d
II-1	1.32 d	58.03 cd	69.0 d	19.7 a
III	1.27 d	75.94 ab	82.7 ab	10.0 abc
IV	1.69 b	54.58 cd	76.3 bcd	14.7 ab
V	1.43 cd	61.69 c	72.7 cd	16.0 ab
VI	1.66 b	63.50 c	87.0 a	4.0 cd
VII	1.24 d	79.70 a	79.0 abc	4.7 cd
VIII	1.56 bc	64.70 c	81.0 abc	6.7 bc

Means in the same column followed by the same letter are not significantly different at $\alpha = 0.05$. I: PEG priming; II-1: 2.5×10^{-5} mol/L ABA; III: 2.5×10^{-5} mol/L ABA +2.9×10^{-5} mol/L GA_3; IV: 2.5×10^{-5} mol/L ABA +5.4×10^{-5} mol/L NAA; V: 2.5×10^{-5} mol/L ABA+2.7×10^{-5} mol/L NAA; VI: 2.5×10^{-5} mol/L ABA +2.9×10^{-5} mol/L GA_3+5.4×10^{-5} mol/L NAA; VII: 2.5×10^{-5} mol/L ABA+4.7×10^{-5} mol/L KT+1.4×10^{-5} mol/L NAA; VIII: 2.5×10^{-5} mol/L ABA +2.9×10^{-5} mol/L GA_3+ 4.7×10^{-5} mol/L KT+ 5.4×10^{-5} mol/L NAA.

germination index compared with the control, however only the combined treatment with GA_3+NAA (Ⅵ) significantly increased percentage of normal seedlings.

The effects of combined treatments on seed germination depended on the kind of plant growth regulator used. Combined ABA priming with NAA (Ⅳ and Ⅴ) showed no extra effect on germination index and percentage of normal seedlings compared to treating seeds with ABA alone, however combined ABA priming with GA_3 (Ⅲ) further increased the percentage of normal seedlings and germination index. Seeds treated in ABA+GA_3+NAA (Ⅵ) solution had the highest percentage of normal seedlings, while those treated in ABA+KT+NAA (Ⅶ) were characterized by the highest germination index and shortest mean germination time. These results were even better than that of priming seeds with PEG.

2.3 Relative water absorption

Though the effect of ABA priming on the germination of *Matthiola bicornis* seeds was similar to that of PEG priming, the seed relative water absorptions of the two treatments were quite different. The relative water absorption of seeds primed with PEG was lower than that of the control, while that of seeds primed with ABA, both alone and in combination with other plant growth regulators, were higher than that of the control. This indicated that it was possible to achieve 'priming effect' without an osmoticum.

Further studies showed that the concentration and the sort of plant growth regulators had effects on seed relative water absorption. Generally, the lower the ABA concentration, the higher the relative water absorption (Table 3). The highest relative water absorption appeared in the treatment of ABA in combination with KT+NAA(Ⅶ), while the lowest in combination with 5.4×10^{-5} mol/L NAA (Ⅳ).

3 Discussion

In our study, the results showed that ABA priming significantly reduced mean germination time and increased germination index, and it did not affect percentage of normal seedlings of *Matthiola bicornis*. These results were in accordance with the findings on carrot and tomato seeds reported by Finch-Savage and McQuistan[9,10].

Previous studies indicated that combined plant growth regulators used in treatment with PEG priming further enhanced the benefits of PEG priming on seed germination and seedling emergence[14-16]. Our results also showed that incorporation of GA_3 into the ABA priming solution further increased the percentage of normal seedlings and germination index of *Matthiola bicornis* seeds, and the highest germination index and the shortest mean germination time were obtained in the combination of KT+NAA and ABA treatment. This indicated that incorporation of other plant growth regulators into the ABA priming could further enhance the benefits of ABA priming on seed germination.

ABA priming could improve seed germination and seedling emergence of some vegetable and flower species. This kind of improvement depended on plant species and the concentration of ABA. For instance, the optimal ABA concentrations for treatment of *Callistephus chinensis* cv. Pola and *Matthiola incana* cv. Brillant seeds were 5×10^{-4} mol/L and 1.25×10^{-5} mol/L, respectively (data not presented here). ABA could effectively prevent the bacteria and fungus growth during seed priming treatment, which had advantages for both appraising the priming effect and practical use. Therefore, ABA priming will be an effective method to improve seed germination.

Both osmotic and ABA priming could improve seed germination. However, the mechanisms involved in were different. In osmotic priming, osmoticum such

Table 3 Comparison of the relative water absorption between PEG priming and ABA priming

Treatment*	Control	Ⅰ	Ⅱ-1	Ⅱ-2	Ⅱ-3	Ⅱ-4	Ⅲ	Ⅳ	Ⅴ	Ⅵ	Ⅶ	Ⅷ
Relative water absorption (%)	50.8b	38.7a	78.6def	72.9cd	65.6c	64.0c	78.3def	72.1cd	80.0def	74.7de	85.2f	81.5ef

Means followed by the same letter are not significantly different at $\alpha = 0.05$. *Ⅱ-2: 5.0×10^{-5} mol/L ABA; Ⅱ-3: 7.5×10^{-5} mol/L ABA; Ⅱ-4: 1.0×10^{-4} mol/L ABA; The others are the same as in Table 2.

as PEG were used to control the hydration of seeds in order to permit pregerminative metabolic events to take place and at the same time inhibit radical emergence. In ABA priming, ABA was used to prevent the radical cell wall loosening and there by cell expansion[8] or antagonize the weakening of endosperm restraint radical cells. ABA priming allows seeds to absorb more water to take the physiological and biochemical events, the 'priming effects' might be better.

As a growth inhibitor, does ABA interfere in the pregerminative metabolic event to take place and subsequent seed germination process? Both our results and that of Finch-Savage and McQuistan[10] noted that ABA priming could improve seed germination, therefore, ABA priming was thought not to affect the pregerminative metabolic events, if the proper ABA concentration was used. However, ABA priming had some effects on the seedling development. Finch-Savage and McQuistan[10] found that root length from PEG priming was significantly greater than that from ABA treatment. In our experiment, we also found ABA priming inhibited the main root growth to some extent, but promoted the root hair development. Was it the effect of ABA priming or because not all of ABA was removed from the treated seeds? The true reason was not understood.

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