Original Research

Changes in ABA, IAA, GA3, and ZR Levels during Seed Dormancy Release in *Idesia polycarpa* Maxim from Jiyuan

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Abstract

We analyzed changes in abscisic acid (ABA), indole-3-acetic acid (IAA), Gibberellin A$_3$ (GA$_3$), and trans-Zeatin-riboside (ZR) content in seeds of *Idesia polycarpa* Maxim. Plant hormones have been widely studied for their roles in the regulation of various aspects related to plant development and, in particular, into their action during seed dormancy release. The obtained evidence suggest that the functions of these hormones are not restricted to a particular development stage, and that more than one hormone is involved in controlling various aspects of plant development. Our results will contribute to understanding the role of these hormones during seed dormancy release in *Idesia polycarpa* Maxim. The contents and balance of endogenous hormones in *I. Polycarpa* seeds changed during the chilling treatment. The contents of IAA, GA$_3$, and ZR and the dynamic ratios of hormones were higher in the chilling treatment than in the control. The ABA content was lower than in the control. The chilling treatment released seed dormancy and significantly improved the seed germination rate. The germination rate was significantly negatively correlated with the contents of IAA and ABA, and positively correlated with the following ratios: GA$_3$/ABA, (GA$_3$ + ZR)/ABA, (IAA, GA$_3$, and ZR)/ABA, GA$_3$/ABA + IAA), and (GA$_3$ + ZR)/(ABA + IAA).

Keywords: abscisic acid (ABA), Indole-3-acetic acid (IAA), Gibberellin A$_3$ (GA$_3$), trans-Zeatin-riboside (ZR), *Idesia polycarpa* Maxim

Introduction

*Idesia polycarpa* Maxim. (Flacourtiaceae) is an deciduous tree with a straight, strong trunk, adaptable to a wide range of environmental conditions, fast growth, strong tolerance to cold and drought, and wide distribution. It is often used as a greening and ornamental tree species, and is also used for wood oil production. In fact, it is known as the ‘beautiful tree oil depot’. The germination rate of *I. polycarpa* seeds is very low. Several studies have reported methods to improve the germination rate, for example by removing the waxy outer layer; immersing in alkaline and straw ash water [1-3]; dewaxing, low-temperature, and wet sand treatment [4]; and a low-temperature and wet sand treatment [5]. However, none of these methods was completely successful. Wang found that *I. Polycarpa* seeds have a dormant character [6]. Many factors affect seed dormancy [7].
Plant hormones play a key role in most physiological processes, and a central role in the integration of diverse environmental cues with the plant genetic program and in shaping morphological structures. They are directly involved in seed dormancy release [8-9].

Abscisic acid (ABA) modulates numerous aspects of plant life, including seed dormancy, embryo maturation, and plant responses to different kinds of abiotic stresses such as drought, high temperature, chilling, and salinity. The seed dormancy release process of seeds is controlled by several hormones such as auxins, brassinosteroids, and ABA. In addition, indole-3-acetic acid (IAA), Gibberellin A$_3$ (GA$_3$), and trans-Zeatin-riboside (ZR) play a major role in the regulation of seed dormancy release. However, there is a lack of works on endogenous changes of Idesia polycarpa Maxim, such as ABA, IAA, GA$_3$, and ZR obtained from seeds. The aim of this work was to investigate the changes in endogenous hormone contents in seeds of Jiyuan provenance of I. polycarpa during the release of dormancy under low temperature, and to analyze the relationship between endogenous hormone content and dormancy release. These results provide important information about the seed dormancy release mechanism of I. Polycarpa and provide the theoretical basis and technical support for breeding this important species.

**Materials and Methods**

**Experimental Materials**

Mature, well-filled seeds of I. polycarpa were collected from Jiyuan Manghe Forest, Henan Province, which is on the northern margin of the distribution I. polycarpa in China.

**Experimental Methods**

Moist I. polycarpa seeds were kept in an artificial climate box (LRH-250-GS I, Guangzhou, China) at 5ºC for 60 d and sampled at 0 d, 20 d, 40 d and 60 d. For the control (CK), seeds were kept under natural conditions and sampled at the same times. To test their germination potential, the seeds were placed in Petri dishes (100 seeds per dish) and kept under a variable temperature regime (night temperature 15ºC, daytime temperature 25ºC – 12 h each). Each treatment was replicated three times. The number of germinated seeds was counted every 5 days. High performance liquid chromatography (HPLC) [10] was used to quantify the endogenous hormone contents in all of the seed samples.

**Results and Discussion**

**Effect of 5ºC Treatment on Endogenous Hormone Contents of I. polycarpa Seeds**

**IAA Content**

As shown in Fig. 1, during the 5ºC treatment, the IAA content of seeds first increased, then decreased, then increased. The highest IAA content was at 20 d (318.58 ng/g) and the lowest was at 40 d (267.94 ng/g). The difference in IAA content among different time points during the 5ºC treatment was extremely significant ($p<0.01$). In the CK, IAA content decreased during the 60-day incubation period. The highest IAA content was at 0 d (304.92 ng/g) and the lowest was at 60 d (185.68 ng/g). The difference in IAA content among different time points was extremely significant ($p<0.01$). The IAA content was higher in the seeds in the 5ºC treatment than in those in CK. There was a high IAA content in seeds in the 5ºC treatment and in the CK.

**GA$_3$ Content**

During the 5ºC treatment, the GA$_3$ content decreased, then increased, and then decreased (Fig. 2). The lowest GA$_3$ content was at 20 d (146.75 ng/g) and the highest was at 40 d (188.49 ng/g). The GA$_3$ content did not differ significantly between 0 d and 60 d ($p>0.05$), but differed significantly among the other time points ($p<0.01$). In the CK, the GA$_3$ content decreased over time. The highest GA$_3$ content was at 0 d (304.92 ng/g) and the lowest was at 60 d (110.70 ng/g). The GA$_3$ content differed significantly between 0 and 20 d ($p<0.01$), but not among the other time points ($p>0.05$).

![Fig. 1. Change in IAA content in Idesia polycarpa seeds from Jiyuan during a chilling treatment at 5ºC.](image1)

![Fig. 2. Change in GA$_3$ content in Idesia polycarpa seeds from Jiyuan during a chilling treatment at 5ºC.](image2)
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**ZR Content**

In the 5°C treatment, the ZR content increased over time (Fig. 3). The lowest ZR content was at 0 d (67.73 ng/g) and the highest was at 60 d (123.70 ng/g). The ZR content did not differ significantly between 20 d and 40 d, but differed significantly among the other time points (p<0.05) – especially between 40 d and 60 d (p<0.01). The ZR content decreased over time in CK. The highest content of ZR was at 0 d (67.73 ng/g) and the lowest was at 60 d (15.25 ng/g). The ZR content did not differ significantly between 20 d and 40 d (p>0.05), but differed significantly among the other time points (p<0.01).

**ABA Content**

During the 5°C treatment, the ABA content decreased and then increased (Fig. 4). The highest ABA content was at 0 d (668.40 ng/g) and the lowest was at 40 d (466.78 ng/g). The ABA content did not differ significantly between 20 d and 60 d (p>0.05), but differed significantly among the other time points (p<0.01). In the CK, the ABA content decreased slightly over time. The highest ABA content was at 0 d (668.40 ng/g) and the lowest was at 60 d (617.37 ng/g). The ABA content differed significantly between 20 d and 60 d (p<0.01), but not among other time points (p>0.05).

**Dynamic Balance of Endogenous Hormones**

During the 5°C treatment (Fig. 5), the following hormone ratios increased and then decreased: (IAA+GA₃+ZR)/ABA; GA₃/ABA; GA₃/(ABA+IAA); (GA₃+ZR)/(ABA+IAA). The following hormone ratios increased: (IAA+GA₃)/ABA; (IAA+ZR)/ABA; (GA₃+ZR)/ABA; IAA/ABA; ZR/ABA; ZR/(ABA+IAA).

In the CK, the following ratios showed a decreasing trend: (IAA+GA₃+ZR)/ABA; (IAA+GA₃)/ABA; (IAA+ZR)/ABA; IAA/ABA. The following ratios increased, decreased, and then increased: (GA₃+ZR)/ABA; GA₃/ABA; ZR/ABA; GA₃/(ABA+IAA); ZR/(ABA+IAA); (GA₃+ZR)/(ABA+IAA).

**Effect of 5°C Treatment on Germination Rate of I. polycarpa Seeds at 15/25°C**

From Table 1, the seeds removed from the 5°C treatment at 20 d, 40 d, and 60 d showed germination rates of 80.33%, 90.67%, and 98%, respectively, when

**Table 1. Variance analysis of germination rate of I. polycarpa seeds at 15/25°C after a 5°C chilling treatment.**

| Low temperature treatment time (d) | Germination rate with 5°C treatment (%) | CK germination rate without 5°C treatment (%) |
|-----------------------------------|----------------------------------------|-----------------------------------------------|
| 0                                 | 36.7±1.6dD                             | 36.7±1.6dD                                    |
| 20                                | 80.3±1.6cC                             | 34.7±0.9dD                                    |
| 40                                | 90.7±2.9bB                             | 33.3±2.9dD                                    |
| 60                                | 98.0±0.7aA                             | 33.7±1.6dD                                    |

Note: different lowercase letters in the same column indicate significant difference (p<0.05), and different capital letters indicate extremely significant difference (p<0.01)
germinated at 15/25°C. In contrast, those removed from CK at 20 d, 40 d, and 60 d showed germination rates of 34.67%, 33.33%, and 33.67%, respectively. The highest germination rate was after the low-temperature treatment for 60 d. The germination rates of seeds subjected to the low-temperature treatment were markedly higher than those of seeds in the CK.

The seed germination rate was significantly affected by the duration of the low-temperature treatment ($p<0.01$). In CK, the seed germination rate was approximately 35% at all time points and was not significantly affected by the duration ($p>0.05$).

Correlations between Endogenous Hormone Contents and Seed Germination Rate

Table 2 shows the correlation analysis between the endogenous hormone contents and the germination rate of *I. polycarpa* seeds at 15/25°C after the chilling treatment at 5°C. There was an extremely significant negative correlation between germination rate and IAA and ABA contents. The germination rate was positively correlated with the following hormone ratios: GA3/ABA, (GA3+ZR)/ABA, and (IAA+GA3+ZR)/ABA (Table 3). The germination rate was also positively correlated GA3/ABA+IAA) and (GA3+ZR)/(ABA+IAA) (Table 4).

The amounts and ratios of phytohormones changed in seeds of *I. polycarpa* during the 5°C treatment. The IAA, GA3, and ZR contents were higher in the chilling treatment than in the CK, and the ABA content was lower in the chilling treatment than in the CK. The low temperature treatment released seed dormancy and significantly improved the germination rate, which was significantly negatively correlated with IAA and ABA contents. Germination was also affected by dynamic balance of hormones, and was positively correlated with the following ratios: (GA3/ABA), (GA3+ZR)/ABA, (IAA+GA3+ZR)/ABA, GA3/ABA+IAA), and (GA3+ZR)/(ABA+IAA).

**Effect of IAA on Dormancy Release of Seeds**

Seed germination can be promoted by IAA. A previous study showed that an increase in IAA content led to an increase in seed germination rate [11]. However, other studies have shown that an elevated IAA content can contribute to seed dormancy [12]. Whether IAA promotes or inhibits seed dormancy depends on its concentration and type [13]. Sprouting in the ear of wheat was effectively inhibited by applying IAA solution to *Triticum aestivum* L. at the mature stage [14]. In a seed germination experiment on *Medicago truncatula*, germination was promoted by a low IAA concentration and inhibited by a high IAA concentration, and the sensitivity to IAA varied among different plants [15]. Under high salt conditions, exogenous IAA regulated germination of *Arabidopsis* seeds, and the inhibitory effect became stronger with increasing IAA concentrations [16]. The large amount
of IAA produced by transgenic Arabidopsis iaa M-OX strongly inhibited the germination of its seeds [17]. These findings in other studies indicate that auxin plays an important role in regulating seed germination, and mainly has an inhibitory effect [18]. After ABA, which induces and maintains seed dormancy, IAA is the next most important hormone in seed dormancy [19]. In this study, the IAA content in I. polycarpa seeds showed an increase-decrease-increase trend during the chilling treatment. The germination rate was negatively correlated with IAA content, indicating that a higher IAA content inhibited the germination of seeds during the dormancy release process in I. polycarpa.

Effect of ZR on Dormancy Release of Seeds

The ZR content in I. polycarpa seeds was very low, and the change in the ZR content varied markedly depending on temperature. Under low temperature, ZR can promote...
dormancy release in seeds of *I. polycarpa*. The results of this study showed that the ZR concentration increased and promoted dormancy release in the chilling treatment, but decreased in the CK. Our results showed that increased ZR resulted in an increase in the germination rate of *I. polycarpa* seeds, consistent with the results of Wang’s study on *Asarum heterotropoides*.

**Effect of ABA on Dormancy Release of Seeds**

Seed dormancy and node germination were shown to be controlled by ABA [20]. It has been reported that whether seeds remain dormant or germinate depends on their sensitivity to ABA, rather than their ABA content [21]. Changes in ABA signals were able to release seed dormancy to some extent [22]. Thus, the content and function of ABA, as well as tissue sensitivity to ABA, affect seed dormancy and germination [23]. The ABA content was significantly and negatively correlated with the germination rate of *I. polycarpa* seeds, indicating that a decrease in ABA content helped to release seed dormancy and promote germination [24].

**Effect of Dynamic Balance of Phytohormones on Dormancy Release of Seeds**

Early genetic studies showed that ABA and GA₃ inhibit each other’s synthesis [25]. These two hormones play a decisive role in the process of seed dormancy and germination [26-27]. In another study, whether seeds remained dormant or germinated depended on the relative contents of ABA and GA₃ [7]. In this study, the GA₃ content showed a decrease-increase-decrease trend during the chilling treatment, and the germination rate was positively correlated with the GA₃/ABA ratio. This result indicated that the seed germination of *I. polycarpa* was affected by the co-operative actions of GA₃ and ABA.

Auxin can inhibit germination, similar to ABA. During seed germination, ABA was shown to inhibit hypocotyl elongation via enhancement of the auxin signal [28]. Auxin interacts with ABA in the regulation of seed germination [29]. Auxin was shown to promote bud dormancy of *Marchantia polymorpha* L. [30]. However, under drought conditions, mutual antagonism between ABA and auxin led to the inhibition of lateral root development as a drought-resistance response [31]. This result demonstrated that the relationship between auxin and ABA is not always fixed, and can change during different physiological processes.

After the low-temperature treatment, the seed germination rate of *I. polycarpa* was positively correlated with the following ratios: (GA₃+ZR)/ABA, (IAA+GA₃+ZR)/ABA, GA₃/(ABA+IAA), and (GA₃+ZR)/(ABA+IAA). This result indicated that the germination rate of *I. polycarpa* seeds was affected by the synergistic interactions among endogenous hormones.

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