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TEMPERATURE REGULATION OF PECAN (*CARYA ILLINOENSIS* WANGENH. K. KOCH) SEED GERMINATION MEDIATED BY FOUR ENDOGENOUS HORMONES

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Abstract

There was a little temperature-dependent change in the four endogenous hormones (IAA, GA_3, ZR and ABA) content of pecan (*Carya illinoensis*) seeds. Significant change of endogenous IAA, GA_3 and ZR contents was observed during seed imbibition and after endocarp breaking. It suggested temperature regulation of pecan seed germination is not mediated by the four endogenous hormones contents.

Introduction

Carya illinoensis Wangenh. K. Koch belonging to the family Juglandaceae originated from North America. It is a tree species with important economic value and grown extensively in China for its edible nuts (Zheng *et al.* 2017). The freshly harvested pecan seeds have a little nondeep physiological dormancy (PD) or conditional dormancy. The freshly harvested pecan seed germinate only over a narrow range of temperature. The optimum germination temperature is 3 to 35°C (Van and Dimalla 1976, Dimalla and Van 1977, Xue *et al.* 2017a). The germination percent of freshly harvested pecan seed were about 84% at 30 to 35°C and 48% at 25°C (Xue *et al.* 2017a). The germination per cent of the pecan seed treated by cold stratification for 60 days is about 95 at 25 to 35°C. The conditional dormancy limits the seedlings production, but the temperature signaling pathways in seeds germination are poorly understood.

Seed germination and dormancy are regulated by light, temperature, and hormones (Kim *et al.* 2008, Piskurewicz *et al.* 2009, Park *et al.* 2011). ABA (abscisic acid) and GA_3 are widely identified to be the seed germination and dormancy regulator (Graeber *et al.* 2012, Hoang *et al.* 2014).

ABA is recognized to be a major positive regulator of both an induction of dormancy and a protector of the dormant (Finch-Savage and Leubner-Metzger 2006, Tan *et al.* 2018). GA_3 is another important hormone, which breaks seed dormancy and causes germination (Graeber *et al.* 2012). Seed dormancy may depend on the balance of ABA and GA_3 (Finch-Savage and Leubner-Metzger 2006). ABA and GA_3 are an important regulator of thermoinhibition of lettuce seed germination (Takeru *et al.* 2004).

IAA is a well-known regulator of plant growth and development, and may be a regulator of seed dormancy and germination (Zhao 2010). Exogenous IAA inhibit wheat seed germination (Ramaih *et al.* 2003). The inhibition of ABA on embryonic axis elongation during seed germination is mediated by IAA (Belin *et al.* 2009, Liu *et al.* 2013). Zeatin riboside (ZR) is identified as endogenous cytokinins in dry *Zea mays* seed (Hocart *et al.* 1988) and previous study showed cytokinins had a positive effect on seed germination (Radomirka *et al.* 2006).

Endogenous IAA, GA_3, ZR (zeatin riboside), and ABA during pecan seed germination at three temperatures using enzyme-linked immunosorbent assay were quantified. This work will add

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current understanding of endogenous hormones in temperature regulation of pecan seed germination.

Materials and Methods

Pecan (*Carya illinoensis* Wangenh. K. Koch) 'Jinghua' seeds produced in Sihong County, Jiangsu Province, China were provided by Jiangsu Academy of Forestry in 2018. Freshly harvested seeds dried and stored in a dry and well-ventilated room and test the seed viability as Xue *et al.* (2017b).

Seeds of similar size were selected for seed germination treatment. Three biological replicates were used and 50 seeds were included in each replicate.

Seeds were placed in water for 10 days at room temperature (15 - 25℃), the seeds were collected at 0, 5 and imbibition 10 day stages. After imbibition, seeds were transferred to germination boxes with wet cotton, and placed in an incubator at 15, 25 and 30℃ for 30 days under dark conditions for three replicates. The seeds were collected at different germination stages (2, 4, 6 and 12 day after imbibition and two special stages: seed endocarp breaking and seed radicle protrusion).

The changes of seed moisture content were tested (determined gravimetrically from 3 × 10 g kernel by weighing kernel before and after drying in an oven (103℃) for about 24 hrs (Xue *et al.* 2018) and the germinated seeds were recorded to calculate the seed germination rate.

About 0.5 g fresh weight (FW) of samples was taken, and the endogenous IAA, GA_3, ZR and ABA were extracted following previous research (Liu *et al.* 2016). The samples were homogenized with 80% (v/v) methanol (5 ml), which contained 1 mmol/l butylated hydroxy-toluene (BHT). The extracting solution was passed through Chromosep C^{18} columns (C^{18} Sep-Park Cartridge, Waters Corp. Millford, MA, USA), and the fractions were vacuum dried at 40°C and dissolved in 1 ml of phosphate-buffered saline (PBS) containing 0.1% (v/v) Tween 20 and 0.1% (w/v) gelatin (pH 7.5) for enzyme-linked immunosorbent assay (ELISA). The quantification of IAA, GA_3, ZR and ABA was performed by ELISA as formerly explained by Liu *et al.* (2016).

The data were analyzed using SPSS 20.0. The means were tested by the least significant difference method at $p < 0.05$ (LSD = 0.05).

Results and Discussion

The absorption of seed moisture can accelerate seed metabolism and affect hormones by seed upon imbibition. The water content of pecan seed kernels was only 4% at first. After 10 day of water absorption, the water content of the seeds was stable at 28%. The change of seed moisture content in 15, 25 and 30℃ did not have a significant difference and temperature did not have a significant effect on the seed moisture content. After seed endocarp breaking, the water content increased rapidly again, the seed water content when seed endocarp breaking was 26% and seed water content when seed radicle protrusion was 46% (Fig. 1).

No seed germinated at 15℃ and only 4% seeds germinated at 25℃ in 22 days. But at 30℃, the seed germination rate was 22% on the 14th day (i.e, placed in the incubator for 4 days), and on the 22nd day, the germination rate was 86% (Table1). These results showed that temperature affect the pecan seed germination significantly and the germination percentage had significant difference at three temperature.

During seed development, endogenous ABA will accumulate continuously, preventing seed germination by inducing seed dormancy. However, during the seed germination progress, the content of ABA continued to decrease, while the content of GA_3 increased continuously during the process of water absorption and stratification (Kai *et al.* 2015).

Table 1. The seed germination rate of pecan seeds germinated in different temperatures.

Temp./°C	Time/days	Germination rate (%)
Room temp.	0	0
	5	0
	10	0
15	12	0
	14	0
	16	0
	22	0
25	12	0
	14	0
	16	0
	22	4
30	12	0
	14	22
	16	37
	22	86

In the progress of pecan seed germination, the endogenous GA_3 are shown in Figs 2 - 3. The GA_3 contents of fresh and dry weight had significantly change, GA_3 content showed a trend of sharp rise following sharp decline during the imbibition stage (0 to 10 days). Although there was a significant difference in GA_3 content of fresh weight (Fig. 2) at 15, 25 and 30°C, the change was small, and the GA_3 content of dry weight was not significantly different at 15, 25 and 30°C (Fig. 3). It showed that different temperatures did not cause differences in the hormone metabolism of pecan seed endogenous GA_3, the difference in GA_3 content was of fresh weight only due to the difference in moisture content of the seed kernel. The GA_3 content of fresh and dry weight increased sharply when seed endocarp broken and seed radicle protrusion took place.

In the progress of pecan seed germination, the endogenous ABA are shown in Figs 4 - 5. During the imbibition days, the content of ABA did not change significantly, but the ABA content of fresh weight decreased because of the seed moisture content increased. Although there was a significant difference in ABA content of fresh weight (Fig. 4) at 15, 25 and 30ºC, the change was less and the ABA content of dry weight was not significantly different at 15, 25 and 30ºC (Fig. 5). It showed that different temperatures did not cause differences in the hormone metabolism of pecan seed endogenous ABA, the difference in ABA content is of fresh weight only due to the difference in moisture content of the seed kernel. Both the endogenous ABA content kept stable when the seed endocarp broken and seed radicle protrusion took place.

Fig. 6 showed that the balance of ABA/GA_3 decreased when seed endocarp breaking. The balance of ABA/GA_3 has a significant effect on seed germination (Kai *et al*. 2015).

In the progress of pecan seed germination, the content of endogenous IAA changed relatively smoothly the endogenous IAA (Figs 7 - 8). During the 0 to 10 day,. During the imbibition days, the content of IAA rises slowly. And the content of IAA fluctuated at 15, 25 and 30°C. The IAA contents of fresh weight (Fig. 7) under the three temperature conditions were significantly different at 12, 14, 16 and 22 day, but the IAA content of dry weight was only significantly different at 20 day and the IAA content at 25℃ was significantly higher than 15℃ and 30ºC. The IAA content of fresh and dry weight increased sharply when seed endocarp broke and seed radicle protrusion happened.

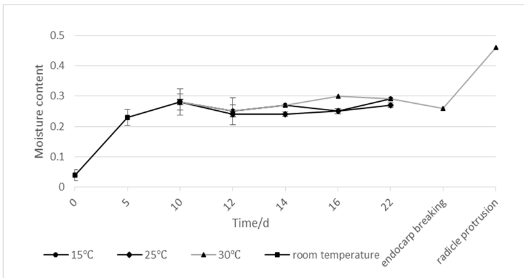

Fig. 2. Changes of GA_3 content of fresh weight during pecan seeds germination at different temperatures (FW means fresh weight).

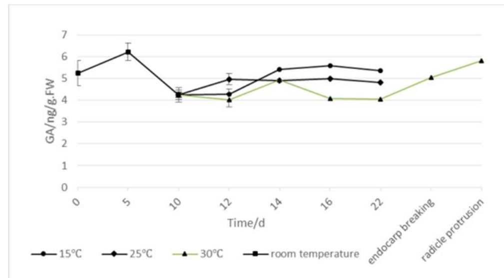

Fig. 1. The moisture content of pecan seeds germinated at different temperatures.

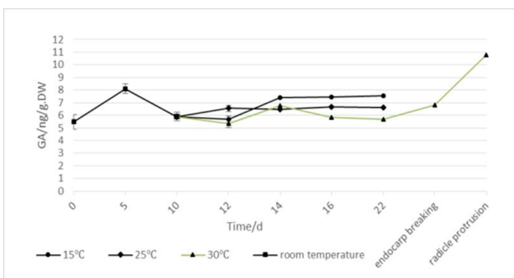

Fig. 4. Changes of ABA content of fresh weight during pecan seeds germination at different temperatures (FW means fresh weight).

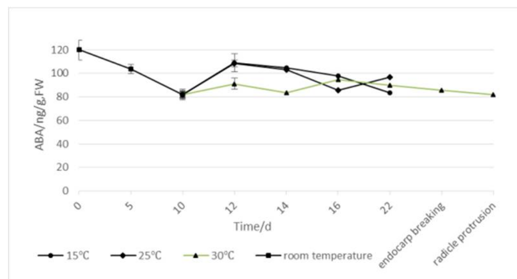

Fig. 3. Changes of GA_3 content of dry weight during pecan seeds germination at different temperatures (DW means dry weight).

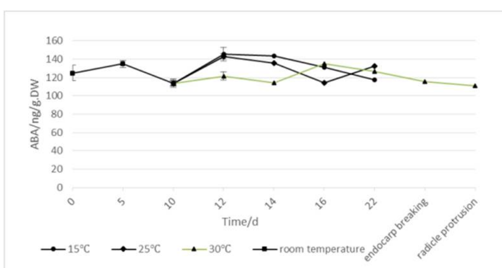

Fig. 6. Changes of the balance of ABA and GA_3 during pecan seeds germination at different temperatures.

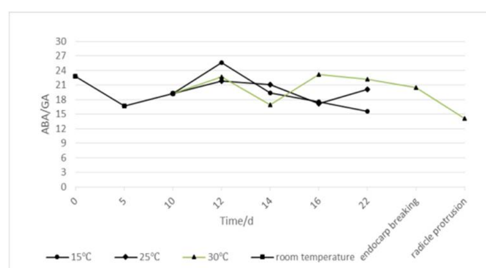

Fig. 5. Changes of ABA content of dry weight during pecan seeds germination at different temperatures (DW means dry weight).

In the progress of pecan seed germination, the endogenous ZR are shown in Figs 9 - 10. During the 0 to 22 day, the content of endogenous ZR changed relatively smoothly. During the imbibition days, the content of ZR did not change significantly. And the content of ZRt showed a trend of sharp rise and then sharp decline at 1, 2 and 30ºC.

The ZR contents of fresh and dry weight (Figs 9 - 10) under the three temperature conditions were significantly different at 14 and 16 day, and the ZR content at 15ºC was significantly higher than 2 and 30ºC. Both the ZR content of fresh or dry weight increased sharply when seed endocarp broke and seed radicle protrusion took place.

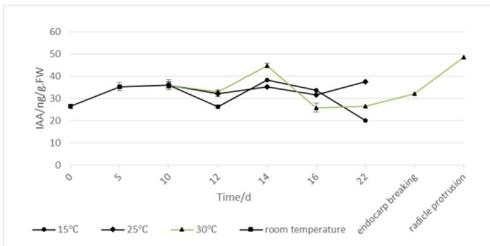

Fig. 8. Changes of IAA content of dry weight during pecan seeds germination at different temperatures (DW means dry weight).

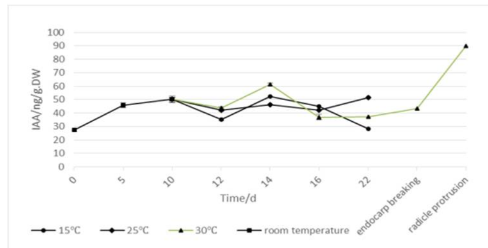

Fig. 7. Changes of IAA content of fresh weight during pecan seeds germination at different temperatures (FW means fresh weight).

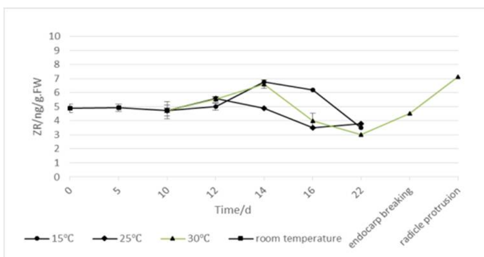

Fig. 9. Changes of ZR content of fresh weight during pecan seeds germination at different temperatures (FW means fresh weight).

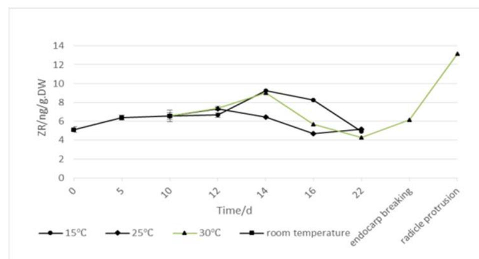

Fig. 10. Changes of ZR content of dry weight during pecan seeds germination at different temperatures (DW means dry weight).

Seed germination is considered to be the most sensitive of all stages of plant growth and is influenced by a variety of environmental stresses. Seed germination in many plant species it is influenced by temperature. High temperature often causes damage to the germinated seeds (Piramila *et al.* 2012, Hasanuzzaman *et al.* 2013), but high temperature promotes pecan seed germination, although germinated pecan seeds need to be removed to a lower temperature (about 15 - 25ºC) for the growing of seedlings.

Model for the regulation of dormancy and germination by ABA and GA_3 in response to the environment (Finch-Savage and Leubner-Metzger 2006) suggest that ambient environmental factors (e.g. temperature) affect the ABA/GA_3 balance and the sensitivity to these hormones and the germination seed with nondeep PD is influenced by ABA and GA_3. Plant hormones have great impact on regulating seed germination (Muller *et al.* 2006). Studies indicated that ABA has an effect on delaying the radicle expansion, which restrained seed germination (Graeber *et al.* 2010). GA_3 has an antioxidant effect on ABA (Miransari and Smith 2014), GA_3 can promote the production of α-amylase, thereby promoting seed germination (Yamaguchi 2008, Liu *et al.* 2016). ABA/GA_3 balance is very important during seed germination. Two major features of the ABA/GA balance were worth considered: the balance of hormone levels and the balance of the signaling cascades. ABA was reported to be involved in the inhibition of GA biogenesis, and GA_3 negatively affects ABA biogenesis during seed germination too (Seo *et al.* 2006, Shu *et al.* 2013). In addition, auxins and cytokinins also play an important role in regulating seed germination, and cytokinins are active throughout the seed germination process (Riefler *et al*. 2006, Kai *et al.* 2015, Liu *et al.* 2016). ZR promotes RNA and protein synthesis by regulating cytokinesis (Satendra *et al.* 2016).

Therefore, is regulation temperature of pecan seed germination mediated by hormones? However, it does not support this hypothesis that there was little temperature-dependent change in the four endogenous hormones contents in pecan seeds. The results provide more ideas for understanding the temperature signaling pathways in seeds germination and the relationship between seed dormancy and germination.

In addition, significant increase of endogenous IAA, GA_3 and ZR contents was observed after endocarp breaking. It suggests the key point of developmental physiological switch from the germination to the seedling program is an endocarp breaking but radicle protrusion. And, the seed endocarp breaking was about 12 hrs earlier than seed radicle protrusion.

There was little temperature-dependent change in endogenous ABA, GA_3, IAA and ZR contents in pecan seeds. Significant increase of endogenous IAA, GA_3 and ZR contents was observed after endocarp breaking. Significant change of endogenous IAA and GA_3 contents was observed during seed imbibition. It suggested temperature regulation of pecan seed germination is not mediated by the four endogenous hormones contents.

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