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南方红豆杉种子综合处理过程中内源激素的动态变化

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摘 要: 为揭示南方红豆杉种子内源激素与休眠的关系,采用酶联免疫吸附法(ELISA)测定了经过层积处理的种皮和胚乳的脱落酸(ABA)、赤霉素(GA3)、吲哚乙酸(IAA)、玉米素核苷(ZR)4 种内源激素含量的变化情况。结果表明:种子胚乳中内源 ABA 的含量随着层积时间的延长而逐渐下降,GA 含量增加,IAA 和 ZR 的含量先增加后降低,GA/ABA、IAA/ABA 和 ZR/ABA 逐渐增大,休眠随之解除。种皮中内源 ABA、GA、IAA 和 ZR 的含量均随着层积时间的延长而逐渐下降。其中 GA/ABA 的变化较大,因此,南方红豆杉种子休眠的限制因子很可能是 ABA 和 GA 的平衡调控作用。

关键词: 南方红豆杉; 种子; 内源激素

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Dynamic changes in endogenous hormones in *Taxus chinensis* var. *mairei* seed during stratification

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Abstract: In order to characterize relationship between endogenous phytohormones and dormancy of *Taxus chinensis* var. *mairei*, changes in contents of four kinds of endogenous phytohormones, i. e., abscisic acid(ABA), gibberellin (GA3), indole acetic acid(IAA) and zeatin riboside(ZR) in spermoderm and endosperm of *Taxus mairei* were analysed by enzyme-linked immunosorbent assays(ELISA). The results showed that ABA contents were gradually decreased in endosperm during stratification, while GA contents were increased and IAA and ZR contents firstly increased and then decreased. Calculated ratio data of GA/ABA, IAA/ABA and ZR/ABA exhibited gradually increasing trends within stratification time and dormancy was relieved accordingly with them. In spermoderm, endogenous ABA, GA, IAA and ZR contents were gradually decreased through stratification. The change in GA/ABA ratio was greater than the other two ones. So it was suggested that the tradeoff between ABA and GA contents could control the dormancy process in *T. chinensis* var. *mairei* seed.

Key words: *Taxus cinensis* var. *mairei*; seed; endogenous hormones

Taxus chinensis var. *mairei* is one of rare Taxaceae *Taxus* species, widely distributing in China, such as in Yangtze River basin, Henan Nanling Mountains, and mountains or valleys in Shanxi, Gansu and Taiwan Provinces. It has attracted global attention for extracting significant anticancer activity of taxol from barks,

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twigs, leaves and other organs(Li *et al.*, 2003; Yuan *et al.*, 2002a, b, c). However, the species have faced strong pressures of utilization in recent years and indeed suffered devastated looting. Thus, biologists were seeking to increase the *T. chinensis* var. *mairei* population within a framework of forest resource management and genetic conservation. *T. chinensis* var. *mairei* seeds with morphophysiological deep dormancy have underdeveloped and dormant embryos, which must grow continuously to some time before seed dormancy could be broken. Listed as one of China's first class key protected wild plants in 1999 (Cao & Chen, 1999), *T. chinensis* var. *mairei* has an endangered existing state because of its low natural reproduction. In recent years, reports on artificial propagation and cultivation of *T. chinensis* var. *mairei* research were both rare in China and other countries, while research on suitable germination conditions was rudimentary. The development and utilization of *T. chinensis* var. *mairei* were seriously restricted by its low seed germination rate. So, it was necessary to study physiological processes of *T. chinensis* var. *mairei* seed germination. Involved in the process of plant growth in all physiological regulations, plant hormone was one of the most important substances in controlling plant growth and development(Ge, 2004). Previous studies on endogenous hormones were mainly concentrated on asexual reproduction(Han *et al.*, 1993; Tan *et al.*, 2008)and physiological resistance(Rock *et al.*, 1999; Shi *et al.*, 2006; Lan *et al.*, 2006; Wang *et al.*, 2009; Zhang *et al.*, 2009). At present, there are many studies on species endogenous hormones, especially on endogenous hormone as a signal molecule involved in regulation of plant adaptation under stress(Quan *et al.*, 2003). In order to carry out in-depth study in this experiment, the paper has taken a combination of temperature and hormones to break the seed dormancy of *T. chinensis* var. *mairei* and discussed changes of several hormones in seed germination process. These results will be helpful to understand the occurring physiological and biochemical processes during alternating temperature stratification and provide reference information of *T. chinensis* var. *mairei* seeds stratification for further study.

1 Materials and methods

1.1 Experimental Materials

T. chinensis var. *mairei* fruits consisting of scarlet or green cuplike arils were collected in Xiushui County, Jiujiang City, Jiangxi Province. With the age of 20 —40 a, *T. chinensis* var. *mairei* grew in 400—500 m sea level of the evergreen and deciduous broad-leaved mixed forest in the valleys and the slopes. Arils and empty seeds were floated off after they were collected and then macerated in water. After naturally dried, the seeds were sealed into polyethylene bags and stored in refrigerator. Experimental materials were fresh seeds. TGW was 65.048 g.

1.2 Experimental design and sampling

Six treatments labeled as A0 to A5 were set in this experiment. Air-dried seeds A0 were soaked in 25 ℃ warm water for 48 h; A1, A2 and A3 were soaked respectively with 200, 500, 1 000 mg · L^{-1} GA3 for 48 h; A4 were washed by water for 1 week. Followed by above treatments, all seeds were mixed with moist sands at the ratio of 1 : 3 for stratification. The seeds were firstly placed under a condition of variable temperature of 23 ℃/10 ℃(12 h light)warm stratification for 4 months, and then placed in 5 ℃ cold stratification for 4 months. A5 were soaked in 25 ℃ water for 48 h, and then mixed with moist sand and placed outdoors for natural stratification for 8 months.

All seeds were randomly sampled every two months during stratification, and placed into -30 ℃ refrigerator. In order to determine the hormones content, seeds were peeled into seed coats and endosperm (including embryos. *Taxus* embryo was usually small and difficult to be separated from endosperm, so the embryos were not separated to test hormones) before hormones were tested. (A3 seeds were almost rotten after stratification, so their hormones were not tested).

1.3 Experimental methods

The seed materials that preserved in refrigerator were weighed for 0.3 g, and added into 2 mL sample extraction. These materials were grinded into homogenate in ice bath and transferred to 10 mL tube. The

mortars were washed with 2 mL extraction and transferred into the tube, then shaken evenly and placed into 4 ℃ refrigerator to extract for 4 h. Supernatant was collected after centrifugation of 4 000 rpm for 15 min. Then 1 mL extraction was added into precipitate and extracted for 1 h at 4 ℃ and centrifuged again. The supernatants were pooled and its volume was recorded. The liquid was processed over C-18 solid-phase extraction column with a process of balance column with 1 mL of 80 % methanol loading samples, washing column with 5 mL of 100% methanol and eluting hormones with 100% ethyl ether(5 mL)and 100% methanol(5 mL). Samples of washed-column were transferred into 5 mL plastic centrifuge tube. Vacuum was concentrated to dry with nitrogen to remove methanol of extraction, and then diluted to constant volume(usually about 1.5 mL samples to 1 g fresh sample dilution constant volume). Endogenous hormones ABA, GA3, IAA, ZR were measured by enzyme-linked immunosorbent assay(ELISA)(Zhang *et al.*, 1991; Wu *et al.*, 1988) Enzyme immunoassay kit was purchased from China Agricultural University. Every sample was repeated for three times by the Spectra Max Plus.

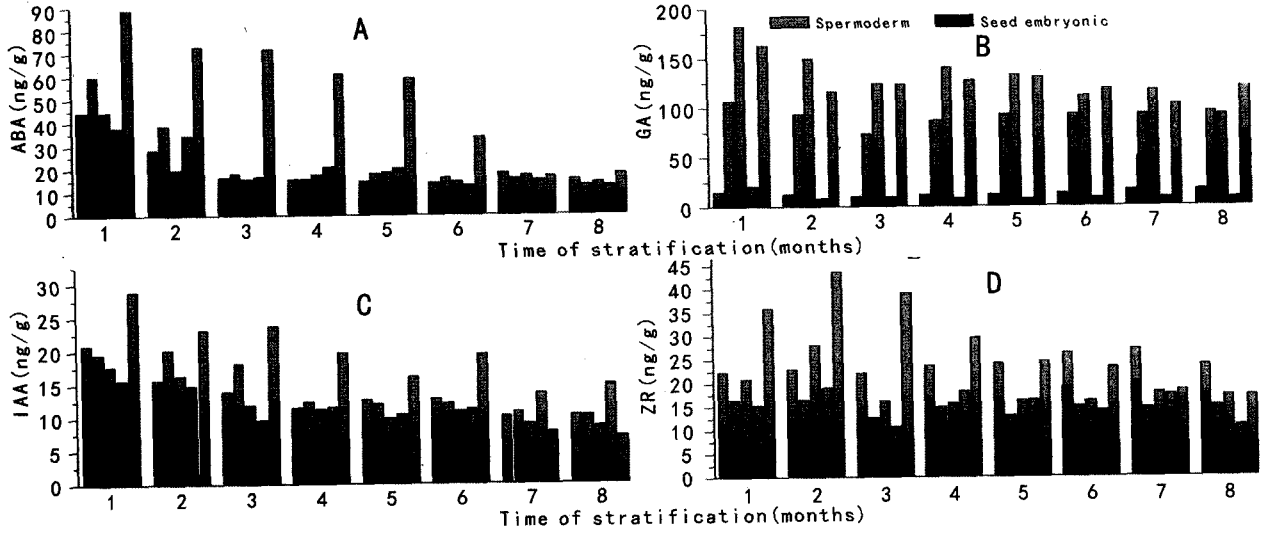

Fig. 1 The changes of hormones content of different treatments in spermoderm and embryonic from *T. chinensis* var. *mairei* seed

histogram of figures from left to right is: A0, A1, A2, A4, A5 and the same follow

2 Results and Analysis

2.1 Change in endogenous hormones content

2.1.1 Change in ABA content ABA could induce dormancy of developed seeds and inhibit embryo germination, but how regulation was carried out remained unknown(Sun *et al.*, 2004). ABA content of seeds in treatments rapidly decreased at first during stratification and then maintained at a low level(Fig. 1:A). The results showed that ABA content was high in mature endosperm and this might be one of the main reasons of seed dormancy. ABA content of endosperm in A0 and A4 treatments decreased slowly, while changes in ABA contents with the other three treatments were obvious, and the change in A5 treatment was the most obvious. ABA synthesis was blocked with the embryo after-ripening, leading to a decrease in ABA content which benefited to the relieve seeds dormancy. There was significant difference by ANOVA of treatments, stratification and their interaction effects. The ABA content was significantly different by LSD among five treatments. Different treatments affected ABA content of endosperm. The differences of ABA contents between March and May, April and May, June and July were not significant by LSD of treatments and stratification, while the differences in other stratification were significant.

The ABA content of seed coats decreased at dif-

ferent speeds during stratification in these treatments (Fig. 1:A). The ABA contents of seed coats were significantly lower in A0, A1, A2 and A4 than in A5. These results showed ABA content of seed coats during stratification continuously decreased. The decrease in ABA content was significantly faster in A0, A1, A2 and A4 than in A5, which benefited to seed germination. According to the decreasing speed of ABA content, the A0, A1, A2 and A4 treatments could be divided into two stages: the first 2—3 months were a phase of sharp decrease and then a stable phase afterwards. ABA content increased in A4 during stratification of 4 — 5 months. ABA content in A5 rapidly decreased during 1—2 months, then slowly decreased during 3—5 months, and then rapidly decreased during 6 — 7 months, and finally lasted in stabilized stage. In the early stratification, the ABA content in seed coat was significantly higher than that in endosperm. Changes in ABA content in seed coat and endosperm were few after stratification for 6 months. ANOVA showed that there were significant differences in ABA content among the treatments, stratification time and their interactive effects. The LSD result showed there was no significant difference in ABA content between A0 and A4, A1 and A4, during 3 to 5, 7 to 8 months. The differences of others' ABA content were significant.

2.1.2 Changes in GA3 content　GA3 was one of the strong active GAs, and could promote cell elongation and break GA3 which played an important role in the promotion of seed development and germination regulation. The GA3 contents of endosperm in different treatments were shown in Fig. 1:B. GA3 content increased slowly in A0, but rapidly in A1 during stratification for 2 months, and remained high in A2. Due to the fact that A1 and A2 seeds were soaked into high concentration of exogenous, the content of GA3 was high. GA3 content firstly increased and then decreased in A4. GA3 content was higher in A5 than others, but the reason was still unclear. ANOVA showed that differences were significant among treatments, stratification and their interaction. Multiple comparisons showed that the differences among treatments were significant, indicating that the effect of exogenous GA3 on endosperm GA3 content in endosperm was obvious. Multiple comparisons of different stratification times showed that the difference of GA content was not significant during stratification for 4—7 months, but others' differences were significant.

GA3 content in seed coat was low in A0 and A4 (Fig. 1: B). GA3 content rapidly decreased during stratification for 3 months and then remained stable in A1 and A2. GA3 content of seed coat fastly decreased for 2 months, and then remained stable in A5. GA3 content was higher in seed coat than in endosperm at the beginning of stratification, and then gained opposite result after stratification for a period. A possible reason was that GA3 might transfer from seed coat to endosperm. ANOVA showed that the differences of GA3 contents were significant among treatments, stratification and their interaction effects. The multiple comparative showed that the differences were not significant between A0 and A4, and between stratification for 3 and 5 months, while the others' differences were significant.

2.1.3 Changes in IAA content　The results of IAA content in endosperm were shown in Fig. 1:C. IAA content of endosperm firstly increased and then slightly decreased in treatments during stratification. IAA change was much obvious in A0, from 2.685 ng/g FW during stratification for 1 month to 7.645 ng/g FW for 5 months, and then to 3.888 ng/g FW for 8 months. The change extents were small in other treatments, for example, IAA content remained stable in A5. At the beginning of stratification, IAA content increased due to it has transferred from seed coat into endosperm, which was corresponding to the decreasing of IAA content in seed coat. Embryo cell division continued to strengthen, leading to a decrease of IAA content. ANOVA showed that the differences of IAA were significant between treatments, stratification and their interaction effects. Multiple comparisons in different treatments showed that there were significant differences of IAA between A2 and other treatments. IAA content was high in A2. The difference between A0 and A1 was not significant, but with the other treatments. A5 had the lease IAA content. There were sig-

nificant differences of IAA between A5 and the other treatments. The multiple comparative also showed that there was no significant difference of IAA content during stratification between 1 and 2 months, among 3, 5 and 8 months, and among 4, 5 and 7 months as well. The results showed that IAA content change in endosperm during stratification was not obvious.

IAA content in seed coat decreased during stratification in different treatments(Fig. 1:C). At the beginning of stratification in all treatments, IAA content in seed coat was higher than that in endosperm, and then IAA content decreased in seed coat and increased in endosperm. ANOVA showed that differences of IAA content were significant between treatments, stratification and their interaction effects. Multiple comparisons of treatments showed that there were significant differences between the treatments except between A0 and A4. There were significant differences among stratification periods except between 4 and 6 months. IAA content in seed coats obviously decreased during stratification.

2.1.4 Changes in ZR content Changes of ZR content in endosperm from different treatments were shown in Fig. 1:D. ZR content of endosperm firstly increased and then slightly decreased during stratification in A0, and was 6.50 ng/g FW in A1. ZR contents firstly increased to a peak during stratification for 2 months, and then decreased in A2, A4 and A5. ANOVA showed that the differences of ZR contents in endosperm were significant between treatments, stratification and their interaction effects. Multiple comparisons of treatments showed that there was no significant difference between A1 and A4, A2 and A5, but in A0. The difference of ZR content was significant during stratification between the first and the other months, but was not significant during stratification among 2, 4 and 6 months, and between 5 and 6 months.

ZR content in seed coats decreased gradually during stratification in A0, A1 and A5(Fig. 1:D). Change of ZR content was much obvious and got a peak of 27.37% during stratification for 8 months in A5. A possible reason was that ZR in seed coats was influenced by external environment in nature condition. ZR content increased at first for 2 months, and then gradually decreased for 4 months, and began to increase for the last 2 months. These changes of ZR were as same as GA3 content of seed coat and endosperm. ANOVA showed that there were significant differences of ZR content in seed coat between treatments, stratification and their interaction effects. Multiple comparisons of treatments showed that there were significant differences among the treatments except among A0, A1 and A4. The difference of ZR was significant among stratification periods except between 6 and 7 months, 7 and 8 months. These results showed that stratification affected on ZR content of seed coat at the beginning of the fifth month.

2.2 Changes of endogenous hormones content ratio

2.2.1 Changes of GA/ABA ratio GA/ABA ratio of species endogenous hormones changed with seed dormancy and germination. The ratio of endosperm slowly increased during stratification (Fig. 2: A), which showed that seed dormancy was gradually relieved. The GA/ABA ratio firstly increased, reaching the peak during stratification for 3 months, and then decreased for 4 months, at last increased again for 5 months in A2. The result was consistent with hormonal balance hypothesis, which indicated that seed dormancy was controlled by the interaction of ABA and GA. The GA/ABA ratio in seed coat did not change during stratification in A0 and A4. The change of the GA/ABA ratio in seed coat firstly increased and then decreased in A1, A2, obviously because seeds were soaked by 200, 500 mg/L of exogenous GA3. The ratio gradually increased during stratification in A5.

2.2.2 Changes of IAA/ABA ratio IAA/ABA ratio in endosperm in treatments was shown in Fig. 2:B. The ratio firstly increased and then decreased in A0 and A2, while gradually increased in A1, A4 and A5. The ratio of the seed coat firstly increased and then decreased and finally stabilized in A0, A1 and A2, while increased during stratification in A4. As the lowest one, the ratio change in A5 was slowly decreased during stratification from 1 to 5 months, and then increased and decreased again.

2.2.3 Changes of ZR/ABA ratio ZR/ABA ratio in

endosperm gradually increased during stratification (Fig. 2:C). Change of the ZR/ABA ratio in A0 was the most obvious and reached a peak for 6 months, but changes of other ratios were little. Greatly varying among treatments, ZR/ABA ratio in seed coat firstly increased and then decreased during stratification in A0, A1, A2, A4 and A5.

2.2.4 Change of(GA+IAA)/ABA ratio　The(GA+IAA)/ABA change of endosperm was consistent with the GA/ABA in general(Fig. 2:D). The ratio gradually increased during stratification, but this change was different. The ratio change was small in A0 and A4 but large in A1 and A5. The ratio firstly increased during stratification for 3 months, and then decreased, but slowly increased again in A2. The(GA+IAA)/ABA ratio change in seed coat was consistent with the GA/ABA.

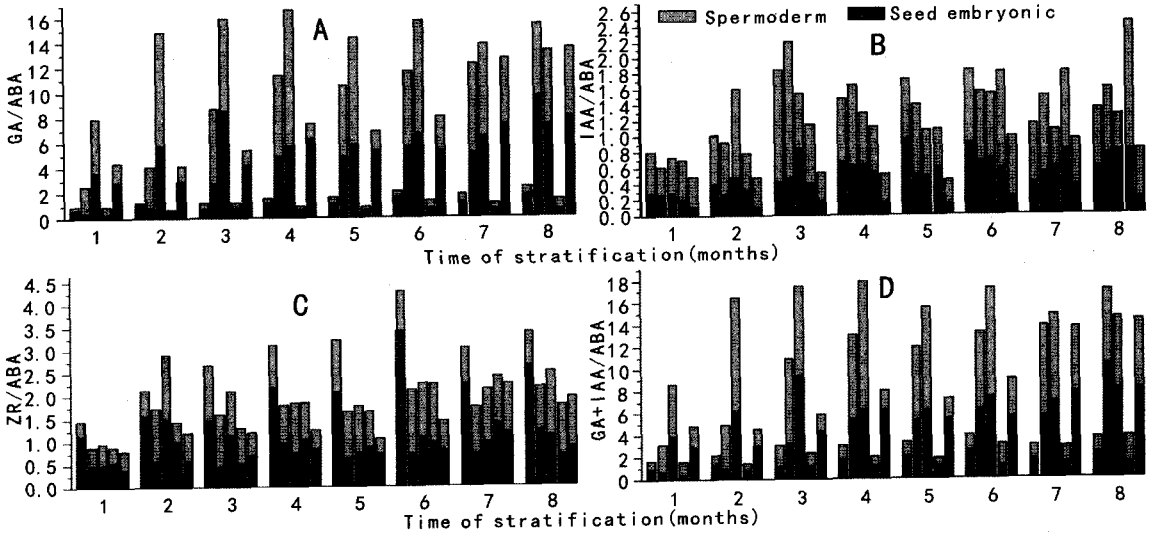

Fig. 2　The changes of hormones ratio among different treatments in endosperm and embryonic from *T. chinensis* var. *mairei* seed

3　Discussion

3.1 Changes of seed's endogenous hormones

The ABA content in seed coat and endosperm rapidly deceased at the beginning of the stratification and then changed a little bit, which showed that the ABA content decreased with the embryo after-ripening at 23 ℃/10 ℃(12 h light)stratification and ABA synthesis was blocked. The IAA content in ABA antagonism increased in the early stratification period and that could promote the degradation of ABA and was beneficial to relieve seed dormancy, which was consistent with predecessors' study(Jacobsen *et al.*, 1985).

The relation of seed dormancy and germination with GA content was noticed wildly (Sun *et al.*, 2006). In this study, during stratification, the GA content of endosperm gradually increased in A0 and A1, which could activate the enzyme activity and promote seed germination. At the early stage of stratification, the GA in seed coat was significantly higher than that in the endosperm. GA content in seed coat decreased during stratification, while its content in endosperm gradually increased. So the GA content in endosperm was slightly higher than that in seed coat. A possible reason was that the part of GA had transferred from seed coat to endosperm during stratification process. So it was suggested that the GA contents could control the dormancy and germination process in seed(Lei *et al.*, 2009).

IAA was a hormone that could promote cell division and expansion, weaken the role of germination inhibiting substances and regulate seed the material of development and energy metabolism (Sheng *et al.*, 2006; Ding *et al.*, 2007; Sha *et al.*, 2007). In general, IAA content in endosperm firstly increased and then slightly decreased, while its content in seed coat gradu-

ally decreased. The IAA content in seed coat was higher than that in endosperm at the early stage of stratification. Then the change of IAA content was little, might because the hormonal part of the seed coat has transferred to endosperm.

ZR concentrations in the seed were relatively high and significantly increased during fruitlet growth (Xiao *et al.*, 2007). In this study, ZR content of endosperm gradually increased during stratification in A0, while the other ZR contents firstly increased and then decreased and finally stabilized. ZR content in seed coat was consistent with endosperm and decreased during stratification. At the beginning of stratification, the ZR content in seed coat was higher than that in endosperm, but then appeared as the opposition.

3.2 Changes of seed endogenous hormones ratio

Research by Khan (1975) illustrated that seed dormancy and germination were related to the absolute content of endogenous hormone in plant and the ratio of various hormones, especially the ratio of hormones that could promote or inhibit growth. The GA/ABA ratio of endosperm increased during stratification, and the biggest increase was 25.6 times in A1. The changes of IAA/ABA, ZR/ABA and (GA + IAA)/ABA gradually increased during stratification. It could be considered as dormancy relieve and germination promotion by increasing IAA/ABA and ZR/ABA in the seed after-ripening process.

The ratio of GA/ABA in seed coat firstly increased and then decreased in A1 and A2 during stratification, while the ratio gradually increased in A5. The IAA/ABA and (GA + IAA)/ABA ratio firstly increased and then decreased during stratification. The ZR/ABA ratio changed during stratification in A0, A1, A4 and A5, but the ratio in A2 firstly increased, then decreased, and at last slowly increased again. The change in GA/ABA ratio was greater than the other two ones. So it was suggested that the tradeoff between ABA and GA contents could control the dormancy and germination process in *T. chinensis* var. *mairei* seed, which results were agreement with previous research on others(Lei *et al.*, 2009).

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