Korean J. Ginseng Sci.
Vol. 20, No. 3, 325~330(1996)

Dynamic Studies on Physiology and Biochemistry in American Seng Seed During Stratification
– Part I. Embryo Ratio, Dry Weight Ratio and Respiration Rate –

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(Received August 29, 1996)

Abstract : Dynamic parameters of physiology including embryo ratio (ER), embryo length to endosperm length, dry weight ratio between embryo and endosperm (DWR) and respiration rate (RR) in American seng (*Panax quinquefolium* L.) seed were investigated. According to the changes of ER during seed stratification, the duration of embryo afterripening could be divided into three stages as ① embryo slow growth stage (ESGS), ER increased from 7.31% to 20.48% (0.16% day^{-1}); ② embryo rapid growth stage (ERGS), ER increased to 80.98% (0.61% day^{-1}) (ESGS + ERGS=morphological afterripening stage (MAS)) and ③ physiological afterripening stage (PAS), ER increased to 88.50% (0.094 day^{-1}) only. DWR increased slowly from 0.20% to 2.76% (0.016% day^{-1}) in MAS and rapidly to 8.81% (0.061% day^{-1}) in PAS. The RR correlated significantly with ER as well ($r=0.8934 > r_{0.01}$ 0.6610). The steep increment of both DWR and RR in PAS indicated that the PAS was not a static stage although the ER was not changed too much. All of these may provide some information for understanding the dormancy mechanisms of American seng seed.

Key words : *Panax quinquefolium*, seed, dormancy, stratification, afterripening.

Introduction

American seng (*Panax quinquefolium* L.) (*Araliacea*) is a valuable and famous natural material for both medicines and health products. It originated from North America and has been introduced and planted in China since 1976.[1] American seng seed has the dormancy property. Germination takes place some 18 to 22 months after seed harvest in natural conditions.[2] This is very helpful for American seng to tide over some harmful environments and propagate its species, but brings many difficulties for us to cultivate it as well.[3] However, little is known about the dormancy mechanisms of American seng.[4,5] Although some endogenous germination inhibitors[6,7] and exo- and endogenous germination promoters[2,8-12] were studied, the dymamic parameters of physiology and biochemistry in American seng seed during stratification have not been reported yet. As we know, before understanding the dormancy mechanisms of American seng seed, it is necessary for us to clarify the dynamic changes of some physiology and biochemistry during embryo afterripening. For this reason, 12 dynamic parameters of physiology and biochemistry, were reported in this series papers including: ER (embryo ratio), DWR (dry weight ratio between embryo and endosperm) and RR (respiration rate) in Paper I; Contents of SC (soluble carbohydrate), CF (crude fat), FA (fatty acid) and SP (soluble protein) in Paper II and POD

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(peroxidase) activity, POD isozymes, ES (esterase) isozymes, RNA and DNA contents in Paper III.

Materials and Methods

1. Seeds

The seeds we used were harvested from four-year-old American seng plants in middle September, 1992 on Huafu Ginseng Farm of Jilin Agricultural University, Changchun, China. The fruit (berries) were hand-harvested, mechanically depulped. The seeds were washed with water and dried in shade. Weight of 1000 seeds was 61.25±0.07 g, water content of seed was 43.34±0.21% and seed vitality by TTC method[13] was 96%.

2. Seed Stratification

The fresh seeds were mixed with mortar sand (1 vol seed/4 vol sand with about 10% moisture) and then stratified in an incubator successively at 20±1℃ (0~80 days), 13±1℃ (81~180 days) and 3°±1℃ (181~260 days).

3. Test of ER

Seed coats of 45 seeds (15×3) were removed and then the seeds were cut into two halves along with the middle line at the seed side. The length of both embryo and endosperm were measured under a binocular microscope.

ER=Embryo length/Endosperm length×100%

4. Test of DWR

The embryo was picked out with a dissecting needle. The seperated embryo and endosperm were put in a drying apparatus at 100℃ for a constant weight.

DWR=Embryo dry weight/Endosperm dry weight×100%

5. Test of RR

The infrared CO_2 analyzer-FQ-W-CO_2 (Analytic Instrument Plant, Guangdong, China) was used for this test.

$$RR=(Ca-Co)/10\times f/22.4\times p/101.33\times 273.15/(273.15+t)\times 1/w\times 1/60$$

Where

RR =Respiration rate ($\mu mol\cdot CO_2\cdot g^{-1}\cdot s^{-1}$)

Ca =CO_2 concentration at gas source ($\mu mol\cdot mol^{-1}$)

Co =CO_2 concentration after gas passing sample room ($\mu mol\cdot mol^{-1}$)

f =Gas flow ($ml\cdot min^{-1}$)

p =Atmosphere (KPa)

t =Temperature in sample room (℃)

w =Sample weight (g)

The parameters of ER, DWR and RR were tested once every 20 days during the seed stratification and all statistical analyses were carried out by using the SYS program (SAU, Liaoning, Chian).

Results and Discussion

1. Dynamic Changes of ER

During all of the afterripening of American seng embryo, the regression between ER and stratification days was not simple straight but curve equation:

$$Y=9.9836-0.3607X+9.55\times 10^{-3}X^2-3.978\times 10^{-5}X^3+4.937\times 10^{-8}X4\ (R^2=0.9922)$$

This result tells us that the growth rate of embryo during the seed stratification correlated significantly with the changes of stratification days and temperature ($r=0.9961>r_{0.01}=0.8610$). As can be seen from Fig. 1, the duration of the embryo afterripening could be divided into three stages:

(1) ESGS (Embryo slow growth stage)

This stage could be called as earlier stage of MAS (morphological afterripening stage) as well, 0~80 days in our experiment with the temperature of 20±1℃. Although the temperature was quite high in this stage, the embryo growth was inhibited by both the germination inhibitors[6,7] and airtight seed coat (will be published soon). ER increased very slowly with the speed of 0.16%

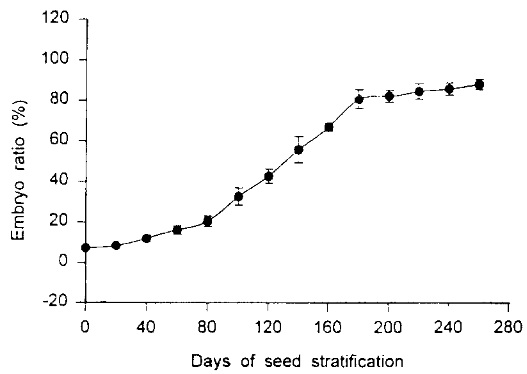

Fig. 1. Dynamic changes of ER during the stratification of American seng seed.
0~80 days (20±1°C)=ESGS
81~180 days (13± 1°C)=ERGS } → MAS; 181~260 days (3±1°C)=PAS.
Embryo ratio was tested once every 20 days.

per day from 7.31% to 20.48%. The regression equation between ER and stratification days in ESGS was:

$$Y=7.7048+7.134\times10^{-2}X+1.236\times10^{-3}X^2 \quad (R^2=0.9958)$$

(2) ERGS (Embryo rapid growth stage)

We also could call this stage as later stage of MAS, 81~180 days in our experiment with the temperature of 13±1°C. Since the seed coat was cracked in this stage, the inhibition caused by airtight seed coat was disappeared. On the other hand, some germination promoters could be formed under the lower temperature.[11,15] We may deduce that the promoters could counteract the effect of germination inhibitors. As a result, the ER increased rapidly from 20.48% to 80.98% with the speed of 0.61% per day, which was 3.8 times as fast as that in ESGS. That is why we call this stage as ERGS. The regression equation was simple straight:

$$Y=-27.7865+0.5980X \quad (R^2=0.9982)$$

Up to here, the embryo of American seng is large enough for its germination, in another word, we may say that the embryo has finished its morphological formation. So that, sometimes, the ESGS and ERGS were put together and called as morphological afterripening stage (MAS).

(3) PAS (Physiological afterripening stage)

After Americam seng seed passing through the MAS, it can still not germination even in a suitable environment. For germination, it has to pass through the PAS under the temperature of 0~5°C.[16] The seed was put in the condition of 3±1°C from 181~260 days in our experiment. Since the seed had finished its morphological afterripening, the increased speed of ER was very slow from 80.98% to 88.50% and 0.094% per day only. The regression equation was:

$$Y=69.6499+6.27\times10^{-2}X \quad (R^2=0.9920)$$

Even though the morphological characteristics and ER of American seng seed was not changed too much during this stage, some physiological and biochemical changes were proved in our studies. This may support us to name this stage as PAS.

From the correlation coefficients in the three stages, $r_{ESGS}=0.9979>r_{0.01}=0.9950$, $r_{ERGS}=0.9991>r_{0.01}=0.9170$ and $r_{PAS}=0.9960>r_{0.01}=9590$, respectively, we may understand that the ER correlated significantly with the stratification days under different temperature. Therefore, we may suggest that the ER could be used as a main index to express the devolopment process of American seng embryo during seed stratification. We may also suggest that we should better use ER instead of the length of embryo in our further research since the dimensions of American seng seeds are quite different. And besides, the stratification days are changed a lot under different methods used by various scientists. For the dormancy mechanism studying of American seng seed, we may suggest again that we should pay attention to the correlation relationship between the paramaters of physiology and biochemistry with ER instead of that with the stratification days.

2. Dynamic Changes of DWR (dry weight ratio)

DWR is a parameter raised by the present authors. As we know, the ER can only express the changes of the embryo length, while many substances are transferred into embryo from endosperm during PAS, but the ER can not reflect it at all. For studying the dormancy mechanism, it is necessary to understand the changes of dry weight during seed stratification, particularly, during PAS.

The regression between DWR and ER was a cubic equation:

$$Y=-1.7053+0.3041X-7.735\times10^{-3}X^2+6.2640\times10^{-5}X^3\ (R^2=0.9761)$$

We can see that DWR correlated significantly with ER ($r=0.9880>r_{0.01}=0.8140$). For this reason, we may deduce that the substances were transferred gradually from endosperm into embryo with the increase of ER.

(1) Slowly increasing stage of embryo weight

As shown in Fig. 2, the changes of DWR could be divided as two stages:

The ER was 7.31~66.97% during this stage. DWR increased slowly from 0.20% to 2.76 with the speed of 0.016% per day, the equation for this stage was:

$$Y=-1.7439+0.3684X-1.502\times10^{-2}X^2+2.765\times10^{-4}X^3-1.768\times10^{-6}X^4\ (R^2=0.9954)$$

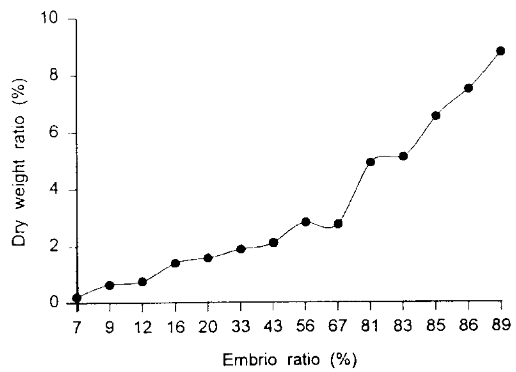

Fig. 2. Dynamic changes of DWR with ER.
7.31~66.97%=Slowly increasing stage of embryo weight
66.97~88.50%=Rapidly increasing stage of embryo weight.
Embryo ratio and dry weight ratio were tested once every 20 days.

This stage was just in the MAS of embryo. The embryo cells were going through the morphological differentiation, most of the substances in endosperm had not transferred into the embryo yet.

(2) Rapidly increasing stage of embryo weight

The ER was 66.97~88.50% during this stage. DWR increased rapidly from 2.67~8.81 with the speed of 0.061% per day. The simple equation for this stage was:

$$Y=-14.8725+0.2548X\ (R^2=0.8447)$$

The embryo weight increased mainly in the PAS of embryo. This phenomenon indicated that most of the substances accumulated in endosperm had been transferred into embryo. Although the ER was not changed too much during PAS, the PAS was not a static stage as well. According to the rapid increase of DWR during PAS, we may infer that PAS is a stage during which many enzymes are activated, particularly, some hydrolytic enzymes in endosperm and synthetases in embryo. And then, the embryo finished morphological afterripening is provided with a large amount of nutrition and energy by endosperm for its germination.

3. Dynamic Changes of RR (respiration rate)

The changes of RR were illustrated in Fig. 3, which showed an increasing tendency during all of the embryo afterripening. The regression equation was:

$$Y=1.6638+7.4910\times10^{-2}X\ (R^2=0.7982)$$

RR correlated significantly with embryo growth ($r=0.8934>r_{0.01}=0.6610$). When ER were 16.20~30.67% and 80.98~82.57%, RR glided down in twice. Except this, RR increased slowly in all other situations. When ER reached to 86.34%, RR rose steeply. This phenomenon tallies with the respiration pattern during the process of relieving seed dormancy. The changes of RR could be divided into five phases as following:

(1) The 1st Phase

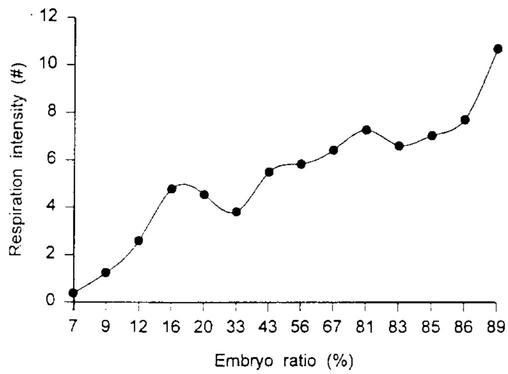

Fig. 3. Dynamic changes of RR with ER
(#)=(10^{-4} μmol CO_2 g^{-1} s^{-1})
7.31~16.20%=The 1st phase(RR from 0.37~4.82 10^{-4} μmol · CO_2 · g^{-1} · s^{-1}),
16.20~32.67%=The 2nd phase (RR from 4.82~3.84 10^{-4} μmol · CO_2 · g^{-1} · s^{-1}),
32.67~80.98%=The 3rd phase (RR from 3.84~7.29 10^{-4} μmol · CO_2 · g^{-1} · s^{-1}),
80.98~82.57%=The 4th phase (RR from 7.29~6.62 10^{-4} μmol · CO_2 · g^{-1} · s^{-1}),
82.57~88.50%=The 5th phase (RR from 6.62~10.72 10^{-4} μmol · CO_2 · g^{-1} · s^{-1}).
Embryo ratio and respiration rate were tested once every 20 days.

RR increased immediately from 0.37 to 4.82 10^{-4} μmol · CO_2 · g^{-1} · s^{-1} when ER increased from 7.31~16.20%. This demonstrated that some enzymes on electron transport chains and TCA (tricarboxylicacid cycle) in chondriosomes were activated.[17] The temperature of 20±1℃ may be advantageous to these enzyme activities.

(2) The 2nd Phase

When ER was from 16.20% to 32.67%, the RR decreased from 4.28 to 3.84 10^{-4} μmol · CO_2 · g^{-1} · s^{-1}. Since the seed was saturated with water, anoxic hypoxia respiration occurred.[18] The inhibitory factor of respiration was the lack of oxygen supply. The inhibition in this phase was related to seed coat and could be decreased by removing the seed coat.[18] The reasonable explanation of anoxic factors in the 2nd phase had not been got yet.[19]

(2) The 3rd Phase

The 2nd respiration height (RR from 3.84 to 7.29 10^{-4} · μmol · CO_2 · g^{-1} · s^{-1}) occurred when ER increased from 32.67% to 80.98%. Since the seed coat was cracked, the oxygen supply was increased. On the other hand, some new chondriosomes and respiration enzyme systems may be formed in embryo cells with the proliferation of embryo.

(4) The 4th Phase

When ER increased from 80.98% to 82.57%, the RR curve glided down from 7.29 to 6.62 10^{-4} · μmol · CO_2 · g^{-1} · s^{-1}. This may be caused by a sudden decrease of temperature which may lead to the activity reduction of some respiration enzymes. Another reason may be that the 2nd respiration system developed before seed germination with a high efficiency may replace the preliminary 1st respiration system with a low efficiency.[20] Therefore, the supersedure between new and old respiration system may induce the temporary reduction of RR.

(5) The 5th Phase

ER were from 82.57~88.50%. This phase was just in PAS of the embryo with the temperature of 3±1℃. The RR increased steeply from 6.62 to 10.72 10^{-4} · μmol · CO_2 · g^{-1} · s^{-1}. The requirement of energy and substances for seed germination may promote the increasing of RR as well as the new respiration system may give expression to a high efficiency.

The changes of RR may point out that the embryo afterripening is a dynamics of physiology and biochemistry.

Conclusions

This study has clearly shown that ① The embryo afterripening of American seng seed during stratification can be divided into three stages as ESGS, ERGS and PAS; ② We suggest that the ER should be used as a main index to express the growth process of American seng embryo; ③ The changes of DWR can be divided as two stages, particularly, the embryo weight increased in PAS can be proved by DWR; ④ The changes of RR indicate that the embryo afterripeing is a dynamics of physiology and biochemistry. The highest RR occurred in PAS tells us that the PAS is not a static stage although the ER is changed little and ⑤ This study also realizes the idea suggested by Proctor *et al.*[5,21] that the germination technology of Oriental ginseng (*Panax ginseng* C.A. Meyer) is adopted for American seng and the seed stratification is finished in 260 days without any exogenous germination promoters.

Acknowledgement

The authors would like to thank the Committee of Science and Technology in Jilin Province, China for their financial support. We gratefully acknowledge the assitance of Prof. De-Hua Ren, Prof. Ying-Chun Ma, Prof. Zi-Yun Lei, Dr. Chang-Zhan Sun and Dr. Zhi-An Zhang in Jilin Agricultural University, Changchun, China and Prof. Gil-Kwan Bae and Prof. Ju-Sik Shin in College of Agriculture, Chungbuk National University, Cheongju, Korea.

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