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Dynamic Studies on Physiology and Biochemistry in American Ginseng Seed During Stratification −Part II. Contents of Soluble Carbohydrate, Crude Fat, Fatty Acid and Soluble Protein−

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Abstract : Dynamic parameters of biochemistry including the contents of soluble carbohydrate (SC), crude fat (CF), fatty acid (FA) and soluble protein (SP) in American seng (*Panax quinquefolium* L.) seed are reported in the present paper. When embryo ratio (ER) increased from 7.31 to 20.48%, the SC content decreased from 4.08 to 1.16%. After that, SC content changed little. The CF content decreased successively from 34.50 to 14.40% from the beginning to the end during the embryo afterripening. The changes of FA content did not correlate with that of ER and the changing range of SP content was not large during the seed stratification. All of these may provide some information for understanding the dormancy mechanisms of American seng seed.

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

Introduction

American seng (*Panax quinquefolium* L.) seed has the dormancy property. It takes place some 18 to 22 months for seed germination after seed harvest in natural conditions.[1] This brings many difficulties for us to cultivate American seng on farms.[2] Unfortunately, up to now, only little is known about the dormancy mechanisms of American seng seed.[3,4] Some endogenous germination inhibitors[5,6], exo- and endogenous germination promoters[7-11] and germination accelerating-technology [12] were reported, but the dynamic parameters of physiology and biochemistry in American seng seed during stratification were studied very few. For understanding the dormancy mechanisms, the dynamic changes of embryo ratio (ER), dry weight ratio (DWR) and respiration rate (RR) of American seng seed during stratification were described in Part I of this series papers.[13] Subsequently, the information about contents of SC, CF, FA and SP is discussed in the present paper.

Materials and Methods

1. Seeds

All of the seeds used in our experiments 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 and 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[14] 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. Soluble Carbohydrate (SC) Content

One gram of endosperm with embryo was homogenized with a little distilled water, fixed to 100 ml and centrifuged (4000 rpm, 10 min). Supernatant fluid (0.5 m*l*) was mixed with 0.5 m*l* redistilled water and 5 m*l* anthrone reagent. Then, the test tube with mixed solution was put into boiling water for one minute and the optical density (OD) was measured at 620 nm with a "722" Optical densitometer (Beijing Analytic Instrument Plant, Beijing, China).

4. Crude Fat (CF) Content

Seeds (20 grains) without seed coat were put in a drying apparatus at 80℃ for 2 hrs and then made into powder. The filter paper and white thread were put in the drying apparatus at 105℃ for 2 hrs as well and weighed subsequently. About one gram of powder was wrapped with filter paper and thread, weighed and extracted at 40℃ with anhydrous ether in a reflux extractor. After drying in the drying apparatus at 105℃ for 2 hrs, the extracted paper parcel was weighed.[15]

5. Fatty Acid (FA) Content

Endosperm with embryo of 20 seeds was homogenized with 3 m*l* 95% EtOH, removed into a test tube and added 22 m*l* EtOH. The test tube was put into hot water at 70℃ for 30 minutes. The filtrate (10 m*l*) was put in a triangle bottle with 2 drops of phenolphthalein and titrated with 0.05 N NaOH until the light red color in the liquid no longer disappeared in one minute. The FA content was expressed by the consumed amount of NaOH for this titration in per gram fresh weight.[16]

6. Soluble Protein (SP) Content

Supernatant fluid (0.1 m*l*, extracted method was the same as that of SC) was mixed with 0.9 m*l* redistilled water and 5 m*l* coomassie blue G-250 reagent and the OD was measured at 595 nm with the "722" Optical densitometer. The calibration curve was made by using BSA (bovine serum albumin).

The parameters of SC, CF, FA and SP were investigated once every 20 days during the seed stratification and all statistical analyses were carried out by using the SYS program (SAU, Liaoning, China).

Results and Discussion

1. Dynamic Changes of SC Content

SC content was high at the beginning and then showed a decreasing tendency during the seed stratification (Fig. 1). The regression equation between SC content and ER was not simple straight but curve equation:

$$Y = 8.4526 - 0.7096X + 2.493 \times 10^{-2}X^2 - 3.523 \times 10^{-4}X^3 + 1.727 \times 10^{-6}X^4 \ (R^2 = 0.8127)$$

SC content correlated significantly with ER ($r = 0.9015 > r_{0.01} = 0.8610$). The respiratory substrates were SC instead of starch and fat.[17]

When ER increased from 7.31 to 20.48% which was just in ESGS (embryo slowly growth stage), the SC content decreased from 4.08 to 1.16%. At the beginning of seed stratification, probably, the activities of some enzymes such as amylase and esterase were inhibited by some germination inhibitors[5,6], some large molecular compounds like starch and fat could not be degraded yet. In this case, the SC was consumed as the main respiratory substrates for the requirement of respiration. This may be the reason for SC content decreasing. However, after that, when ER was from 20.48 to 88.50%, the SC content was not

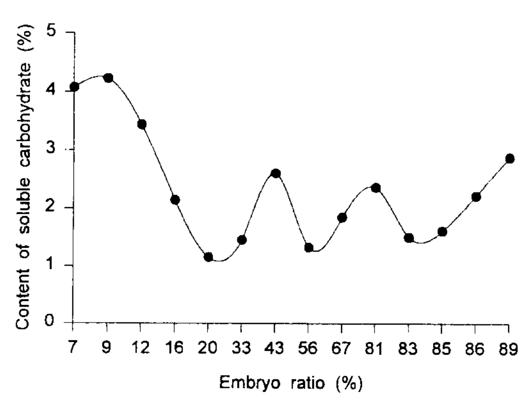

Fig. 1. Dynamic changes of SP content with ER. Embryo ratio and content of soluble carbohydrate were tested once every 20 day.

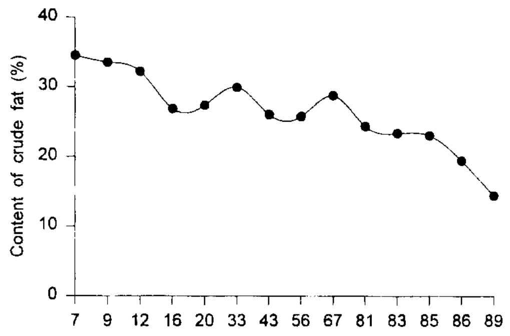

Fig. 2. Dynamic changes of CF content with ER. Embryo ratio and content of crude fat were tested once every 20 day.

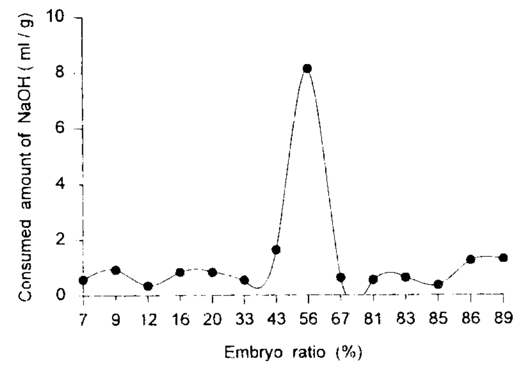

Fig. 3. Dynamic changes of FA content with ER. Fatty acid content was expressed by the consumed amount of NaOH for the titration in per gram fresh weight. Embryo ratio and content of fatty acid were tested once every 20 day.

changed to much, only swung within the range from 1.33 to 2.89%.

2. Dynamic Changes of CF Content

As will be seen from Fig. 2, CF content decreased from 34.50 to 14.40% with the increase of ER during embryo afterripening. The regression between CF content and ER satisfied the exponential equation:

$$Y = 3.3599Xe^{-0.03293X} \ (R^2=0.9349)$$

The negative correlation ($r= | -0.9669 | > r_{0.01} = 0.6610$) existed between embryo growth and CF content. This result suggests that the fat metabolism may play an important role during the afterripening of American seng embryo.

3. Dynamic Changes of FA Content

After the multinomial calculation, the most suitable equation for the regression between FA content and ER was:

$$Y = 5.8291 - 0.8699X - 3.92 \times 10^{-2}X^2 - 6.169 \times 10^{-4} X^3 + 3.05 \times 10^{-6}X^4 \ (R^2=0.4176)$$

The dynamic changes of FA content did not correlate with the embryo growth ($r=0.6462 < r_{0.05}= 0.7860$). Therefore, FA content could not be used as an index for expressing the growth process of embryo during seed stratification. As shown in Fig. 3, at the beginning of the embryo afterripening (ER was from 7.31 to 20.48%), the FA content was low since the CF content decreased only a little (Fig. 2). After that, the FA content increased with the decrease of CF content. However, in this case, ER was only 32.67% and DWR was only 1.90%.[13] The small embryo could still not consume most of the FA. Therefore, the accumulative peak of FA occurred. After that, the ER increased to 66.97% and DWR was 2.76%, this was just in the rapid increasing stage of embryo weight. Most of the FA accumulated were consumed for respiration and transferred into embryo to form some large molecular compounds. This induced that the accumulated peak of FA disappeared steeply. The experiment reported here demonstrated again that the fat metabolism was the main metabolic pathway during the stratification of American seng seed.

4. Dynamic Changes of SP Content

The change of SP content was small only within 3.11~8.31%. The regression equation between SP content and ER was:

$$Y = 7.0927 - 0.1245X + 1.143 \times 10^{-3}X^2 \ (R^2 = 0.2545)$$

The statistics result showed that the SP content did not correlate with the embryo growth ($r = 0.5045 < r_{0.05} = 0.7030$). Just the same as FA content, the SP content could not be used as an index for expressing the dormancy relieving process during the seed stratification either. As can be seen from Fig. 4, the SP content was hight at

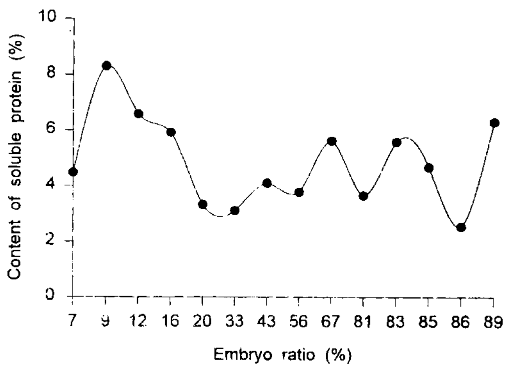

Fig. 4. Dynamic changes of SP content with ER. Embryo ratio and content of soluble protein were tested once every 20 day.

the begining, and then, decreased. The change tendency of SP content in American seng seed during stratification was just similar with that in seed of *Magnolia denudata* Desr.[18]

Conclusions

From these results we may conclude that ① The SC content is high at the beginning and then a dynamic equilibrium occurs during the stratification; ② The CF content decreases from 34.50 to 14.40% with the increase of ER. Fat metabolism is the main metabolic pathway during embryo afterripening of American seng; ③ The FA content can not be used as the index of embryo growth. However, FA content can again demonstrate that fat metabolism is the main metabolic pathway in American seng seed and ④ The SP content can not be used as the index for understanding the dormancy relieving process. The SP content does not correlate with the embryo growth.

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