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Study on Dormancy Mechanisms of American Ginseng Seed II – Germination Inhibition of Seed Coat

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Abstract – This paper gives a description about the germination inhibition of American ginseng (*Panax quinquefolium* L.) seed coat. The existence of seed coat is one of the inhibitory factors which inhibit the embryo growth, particularly during the morphological after-ripening stage. The seed coat can obstruct the water absorption at the beginning of seed stratification, but it can not threaten seed germination. The inhibition of seed coat is not caused by the mechanical fetter neither. However, before splitting the seed coat, the inhibition of seed coat comes from both air-tight character and inhibitors, and after splitting the seed coat, the inhibition may come mainly from the inhibitors.

Key words – American ginseng (*Panax quinquefolium* L.), seed, dormancy, inhibition, seed coat.

Introduction

American ginseng (*Panax quinquefolium* L.) is in the same genus with Oriental ginseng (*Panax ginseng* C.A. Meyer). However, the active components and contents between these two ginsengs are different. The content of ginsenoside Rb1 in American ginseng is significantly higher than that in Oriental ginseng; On the other hand, the content of ginsenoside Rg1 in Oriental ginseng is higher than that in American ginseng. Therefore, these two ginsengs can not be replaced each other in many situations (Li and Xia, 1983). American ginseng originated from North America has been recorded in China since 1757 and planted in China since 1976 (Li, 1980).

American ginseng reproduces by seed. The botanical definition about seed is that: "Seed is a reproductive organ developed from the ovule of higher plant" (Zhang, 1992). In fact, the so-called "seed" of American ginseng as we usually see is not a "seed" but a "kernel" which contains a seed (Lu, 1980). The American ginseng fruit is a berry-like drupe. Its structure from outside to inside are exocarp (rind) → midcarp (pulp) → endocarp (seed coat) → endosperm → embryo cavity → embryo (Cui and Gao, 1988). Nevertheless, for convenience, we still call the midcarp as "pulp", the endocarp as "seed coat" and the kernel as "seed" in the present series papers.

As we know, American ginseng seed has the dormancy property. Germination takes place some 18 to 22 months after seed harvest in natural conditions (Jo *et al.*, 1988). This property is very helpful for American ginseng to tide over some harmful environments and propagate its species, but bring many difficulties for us to cultivate it as well (Huang *et al.*, 1993). However, up to now,

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only a little is know about the dormancy mechanisms of American ginseng seed (Huang *et al.*, 1995b; Proctor and Louttit, 1995) although some germination inhibitors (Huang *et al.*, 1994; Huang *et al.*, 1995c) and hastening methods for germination (Huang *et al.*, 1995a) were reported.

Therefore, the further studies on dormancy mechanisms of American ginseng seed including: **Germination inhibition of pulp** in Paper I; **Germination inhibition of seed coat** in Paper II; **Comparison of inhibitory effects among different fruit parts** in Paper III and **Dynamic changes of germination inhibition during embryo after-ripening** in Paper IV were reported in these series papers.

Experimental

Plant material – All of the seeds used in our experiments were harvested from four-year-old American ginseng plants in mid 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.

Test of water penetration – The seed was dried at room temperature for about one month. After that, three treatments were set up as following:

A=Normal seed: Seed coat was not cut;

B=Half cutting of seed coat: Seed coat was cut along with the side line until half circle and the seed coat was still put on the seed;

C=Removing seed coat: Seed coat was cut along with the side line until complete circle and the seed coat was removed from the seed.

20 seeds in each treatment were weighed accurately, and then put in a culture dish with water. During the first 12 hrs, the seeds were taken out from the culture dish every two hours, the water on the seed surface was removed by filter paper and the seeds were weighed accurately. After 12 hrs, the seeds were weighed every 24 hrs until the seed weight did not increase (Huang, 1986; Lai *et al.*, 1989). Three replications were set up for each treatment.

$WA(\%)=(Wa-Wb)/Wb\times 100$

Where

WA=Water absorptivity(%)

Wa=Seed weight after absorbing water (g)

Wb=Seed weight before absorbing water (g)

Test of air penetration – Four treatments were set up as following:

A=Normal seed: Seed coat was not cut;

B=Half cutting of seed coat: Seed coat was cut along with the side line until half circle and the seed coat was still put on the seed;

C=All cutting of seed coat: Seed coat was cut along with the side line until complete circle and the seed coat was still put on the seed;

D=Removing seed coat: Seed coat was cut along with the side line until complete circle and the seed coat was removed from the seed.

Seeds in different treatments were put in culture dishes with filter paper and water under the room temperature. Ten days later, the respiration rate (RR) was tested with an infrared CO_2 analytic instrument – FQ-W-CO_2 (Analytic Instrument Plant, Guangdong, China) (Li, 1988; Zhou, 1991).

$RR=(Ca-Co)/10\times f/22.4\times p/101.325\times 273.15/(273.15+t)\times 1/w\times 1/60$

Where

RR=Respiration rate (μmol CO_2 $g^{-1}s^{-1}$)

Ca=CO_2 concentration at gas source (μmol mol^{-1})

Co=CO_2 concentration after passing sample room (μmol mol^{-1})

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

p=Atmosphere (Kpa)

t=Temperature in sample room (℃)

w=Sample weight (g)

Effect of seed coat on embryo ratio – Four treatments in this experiment were set up exactly the same as that in the test of air

penetration.

During the first 60 days, the seeds were cultured under the temperature of 20±1℃, and then under 13±1℃. The embryo ratio (ER) of 15 seeds in each treatment were tested every 20 days.

ER(%)=Embryo length/Endosperm length × 100

All statistical analyses in the present paper were carried out by using the SYS program (SAU, Liaoning, China).

Results and Discussion

Water penetration of seed coat – As can be seen from Table 1, after the seed absorbed water from the 8th to the 24th hrs, the water absorptivity (WA) in treatment C (Removing seed coat) and B (Half cutting) were significantly higher than that in treatment A (Normal seed) at 0.01 level. The result here indicates that the seed coat of American ginseng has the effect to obstruct the water absorption at the beginning of Morphological after-ripening stage.

In fact, no matter normal seed (A), half cutting seed (B) or seed removed seed coat (C), all the WA in these three treatments increased sharply during the first two hours (Fig. 1). After that, the WA in treatment B and C still kept a fast increasing tendency until the 12th hrs. However, the one in treatment A went gently after two hours. From Fig. 1, we also can see that, saturation points of WA in treatment C, B and A appeared till the 24th, 72nd and 120th hrs, respectively. This experiment tells us that the saturation point of WA arrived on the first day in seed removed seed coat could also be arrived at the third and fifth days in half cutting seed and normal seed. As we know, the embryo after-ripening takes 18~22 months under natural conditions. While, it takes only five days for normal seed to arrive the saturation point of WA, so that , it could be neglected.

Table 1. Comparison of water absorptivity among different treatments

Time (hrs)	Water absorptivity(%)			P(LSR)
	C	B	A	
2	16.25b	30.21a	25.82ab	<0.05
4	30.09a	34.58a	28.08a	>0.05
6	46.52a	41.76ab	31.54b	<0.05
8	54.92a,A	47.14a,AB	33.41b,B	<0.01
10	61.04a,A	51.98a,AB	35,89b,B	<0.01
12	64.62A	56.21A	38.81B	<0.01
24	66.67A	66.75A	46.31B	<0.01

A=Normal seed; B=Half cutting of seed coat; C=Seed removed of seed coat. Values that corresponded by the same letter (Capital letter: P=0.01; Small letter: P=0.05) in each row were not significantly different from each other; Conversely, values that corresponded by different letter were significantly different at the level indicated by the P valus (F-test and LSR test).

The results obtained in this research are: The seed coat can obstruct the water absorption, but the obstruction could be overcome only after five days and could not threaten the embryo after-ripening. Therefore, we may consider that the dormancy of American ginseng seed is not caused by the water penetration of the seed coat.

Air penetration of seed coat – As shown in Fig. 2, the respiration rate (RR) in treatment D (Removing seed coat) was sig-

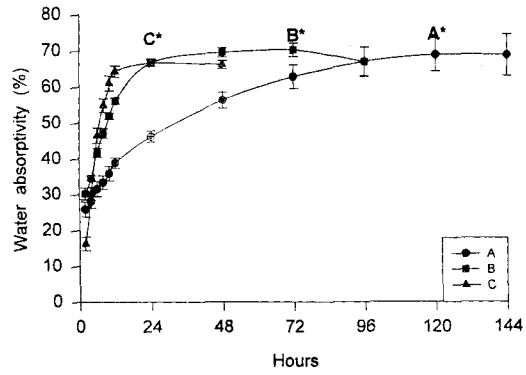

Fig. 1. Water absorptivity curve of American ginseng seed in different treatments. A=Normal seed; B=Half cutting of seed coat; C = Seed removed seed coat. A*=Saturation point of water absorptivity in treatment A; B*=Saturation point of water absorptivity in treatment B; C*=Saturation point of water absorptivity in treatment C.

nificantly higher than those in other three treatments. Particularly, the RR in treatment D 16.54±0.61 μmol CO_2 $g^{-1}s^{-1}$, was four times higher than that in treatment A (Normal seed) which was only 3.73±0.60 μmol CO_2 g^{-1} s^{-1}. From this result, we can understand that the seed coat of American ginseng was really with the obstruction of air penetration.

In addition, between treatment B (Half cutting) and C (All cutting) were not significantly different each other, their RR were 11.56±0.40 and 12.75±0.84 μmol CO_2 $g^{-1}s^{-1}$, respectively. Both treatment B and C were significantly higher than that in treatment A. No matter half cutting seed (B) or all cutting seed (C), the air penetration was increased after the seed coat was cut.

Further analysis made us realize that the inhibition of seed coat to embryo growth was caused not only by the air penetration since the RR in treatment B and C were significantly lower than that in treatment D. If the inhibition was caused only by air penetration, after the seed coat was cut for ten days, the embryo should obtain enough oxygen and the RR in treatment B and C should be at the same level as that in treatment D. Therefore, we may imagine that there may be some other reasons for seed coat to inhibit the embryo growth except the air penetration. We consider that the first reason is that there may exist some germination inhibitors in seed coat and the second reason is that there may exist the mechanical fetter of seed coat. A germination inhibitor in seed coat, acetic acid with IC_{50} (50% inhibitory concentration)=69.18 μl/L, has been verified by the present authors (That will be published in Paper III of this series) and the mechanical fetter of seed coat will be discussed in the next paragraph. Up to here, our experiments confirmed that inhibition of seed coat to embryo growth was caused by both air penetration and germination inhibitors.

Effect of seed coat on embryo afterripening – The changes of embryo ratio (ER) affected by the seed coat were illustrated in Fig. 3. The ER in treatment A, B, C and D were 8.64, 8.49, 8.04 and 8.48%, respectively.

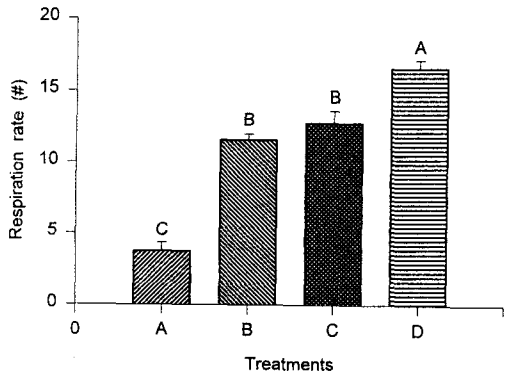

Fig. 2. Respiration rate of American ginseng seed in different treatments. Treatment A=Normal seed; Treatment B=Half cutting of seed coat; Treatment C=All cutting of seed coat; Treatment D=Seed removed of seed coat. (#)= (μmol CO_2 $g^{-1}s^{-1}$). Values that corresponded by the same letter were not significantly different at 0.01 level; Conversely, values that corresponded by different letter were significantly different at 0.01 level. (F-test and LSR test).

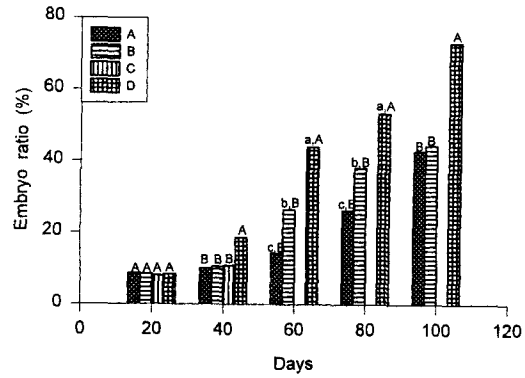

Fig. 3. Effect of seed coat on embryo growth. Treatment A=Normal seed; Treatment B = Half cutting of seed coat; Treatment C=All cutting of seed coat; Treatment D=Seed removed of seed coat. Values that corresponded by the same letter (Capital letter: P=0.01; Small letter: P=0.05) on the same day were not significantly different from each other; Conversely, values that corresponded by different letter were significantly different (F-test and LSR test).

No significantly different among them on the 20th day after stratification.

However, on the 40th day, treatment D (Removing seed coat) with the 18.52% of ER was significantly larger than those in treatment C (All cutting, 10.80%), B (Half cutting, 10.55%) and A (Normal seed, 10.09%). It demonstrated that the seed coat was really with the inhibition to embryo growth. Moreover, we also could deduce that the inhibition of seed coat was not caused by the mechanical fetter. If it was, the seed coat in treatment C should be without any inhibition and the ER should be at the same level as that in treatment D since the seed coat was cut as a complete circle and the seed coat was only put on the seed. In this case, it should be without any mechanical fetter in the seed coat of treatment C. Actually, the ER in treatment C was the same level as that in B and A, and significantly smaller than that in treatment D at 0.01 level.

A similar situation occurred on the 60th and 80th days, the ER in treatment B (26.36 %, 60th day; 37.91%, 80th day) and A (14.24 %, 60th day; 26.10%, 80th day) were significantly smaller than those in treatment D (43.81%, 60th day; 53.17%, 80th day) at 0.01 level; On the other hand, the ER in treatment B was significantly larger than that in treatment A at 0.05 level on the 60th and 80th days. All of these indicated that the seed coat was with inhibition to embryo growth (All of the seeds in treatment C were rotted when the test arrived on the 60th day. The reason is that this treatment (All cutting of seed coat) was polluted by some fungi in the air).

On the 100th day, the ER in treatment B (44.16%) and A (42.57%) were still significantly smaller than that in treatment D (72.87%) at 0.01 level. The seed coat was still with the inhibition to the embryo growth. But, in this period, the seed coat of normal seed in treatment A had split already. It is clear that the inhibition of seed coat is not caused by air penetration after split of seed coat. The only possibility is that the inhibition of seed coat was caused by some inhibitors in the seed coat.

It can therefore be concluded that American ginseng seed has the dormancy characters caused by the obstruction of seed coat. This obstruction is not induced by water-tight character and mechanical fetter of seed coat. However, before split of seed coat, the obstruction comes from both air-tight character and inhibitors and after that, the inhibition may be mainly from inhibitors. So that, we may recognize that American ginseng seed coat is one of the inhibitory factors which inhibit the growth of American ginseng embryo, particularly during the Morphological after-ripening stage.

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References

Cui, D. S. and Gao, Z. S., *American ginseng*, Scientific Press, Beijing, 1984, pp.21.

Huang, Y. G., Cui, S. Y., Li, X. G. and Yang, J. X., Research advances in chemical germination accelerating-technology for Oriental and American ginseng seeds. *Ginseng Research,* **7(3)**, 6-10 (1995a).

Huang, Y. G., Cui, S. Y., Yang, J. X. and Li, X. G., Dormancy causes in and settlement measures for the seeds of wild medicinal plants. *Journal of Jilin Agricultural University,* **15**, 203-207 (1993).

Huang, Y. G., Cui, S. Y., Yang, J. X. and Li, X. G., Preliminary study on inhibitors in American gin-

seng seed. *Journal of Jilin Agricultural University,* **16**, 9-14 (1994).

Huang, Y. G., Cui, S. Y., Yang, J. X. and Li, X. G., Research advances of dormancy mechanism in Oriental and American ginseng seeds. *Ginseng Research,* **7(1)**, 3-7 (1995b).

Huang, Y. G., Li, X. G. and Cui, S. Y., Growth inhibitors in American ginseng seeds. *The Challenges of the 21st Century - Proceeding of the International Ginseng Conference - Vancouver 1994*, 1995c, pp. 397-398.

Huang, Y. G., Study on embryo dormancy of *Kalopanax septemlobus. Journal of Northeast Forest University,* **14**, 39-44 (1986).

Jo. J., Blazich, F. A. and Konsler, T. R., Post-harvest seed maturation of American ginseng: Stratification temperatures and delay of stratification. *HortScience,* **23**, 995-997 (1988).

Lai, L., Zheng, G. H. and Xing, H. W., Relationship between dormancy of Korean pine seed and its seed coat. *Acta Botanica Sinica,* **31**, 928-933 (1989).

Li, J. Y., Preliminary study on dormancy physiology and germination-accelerating technology of lacquer tree seed. *Seed*, (3), 17-19 (1988).

Li, X. G. and Xia, Y. L., Comparison of active constituent between American ginsengs produced in China and North America. *Science and Technology of Chinese Medicinal Materials*, (2), 32-34 (1983).

Li, X. G., Study on active constituent and pharmacological effects of American ginseng. *Special Product Experiment*, (1), 61-62 (1980).

Lu, Y. X., Germination physiology of ginseng seed. *Plant Physiology Communications*, (4), 18-22 (1980).

Proctor, J.T.A. and Louttit, D., Stratification of American ginseng seed: Embryo growth and temperature. *Korean J. Ginseng Sci.,* **19**, 171-174 (1995).

Zhang, C. X., On seed modernization. *Seed*, (5), 43-45 (1992).

Zhou, Y. X., Preliminary study on dormancy physiology of *Manglieta insignis. Seed*, (6), 10-13 (1991).

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