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Inhibition Effects of Pulp on Seed Germination of American Ginseng

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과육이 서양삼 종자 발아에 미치는 영향

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ABSTRACT : The germination inhibitory effects of American ginseng (*Panax quinquefolium* Linne) pulp were discussed. The germination inhibitory effects of pulp juice were decreased in a concentration dependent manner. When the pulp juice was diluted 0 (original juice), 1, 2, 5, 10, 20, 50 and 100 times, the radicle lengths of the assay plant, Chinese cabbage (*Brassica chinensis* Linne), showed 0, 0.32, 0.72, 3.13, 4.83, 16.07, 16.73 and 23.50 mm, respectively (CK=25.98 mm). The pulp evidently inhibited the embryo growth in natural fruit. The longer was the duration that the pulp stayed around the seed, the longer was the time course needed for embryo getting free from the inhibitory effects of pulp. When the depulping was performed on the day 0, 15, 30 and 60 after harvest, the time courses needed for embryo extricating the residual inhibitory effects from pulp were 30, 75, 135 and 135 days, respectively. Moreover, if the pulp stayed around the seed with time, that would make the seed rotten ratio increase. When the pulp stayed around the seed for 0, 15, 30, 60 and 270 days, the seed rotten ratios were 5.47, 5.71, 19.05, 27.14 and 33.33%, respectively. Therefore, we concluded that the pulp could be included in the inhibitory components which made American ginseng seed get into dormancy.

Key words : American ginseng (*Panax quinquefolium* Linne), Seed, Dormancy, Inhibition, Pulp, Chinese cabbage (*Brassica chinensis* Linne).

INTRODUCTION

American ginseng (*Panax quinquefolium* Linne) 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)[12]. American ginseng originated from North American has been

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recorded in China since 1757 and planted in China since 1976 (Li, 1980)[13].

American ginseng is reproduced by seed. The botanical definition about seed is that : "Seed is a reproductive organ developed from the ovule of higher plant." (Zhang, 1992)[16]. 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)[14]. 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, 1984)[2]. Nevertheless, for convenience, we still call the midcarp as pulp, the endocarp as seed coat and the kernel as seed in the present paper.

As we know, American ginseng seed has dormancy. Germination takes place some 18 to 22 months after seed harvest in natural conditions (Jo et al., 1988)[11]. This property is very helpful for American ginseng to tide over some harmful environments and propagate its species, but brings many difficulties for us to cultivate it as well (Huang et al., 1993)[6]. However, up to now, only a little is known about the dormancy mechanisms of American ginseng seed (Huang et al., 1995b; Proctor and Louttit, 1995)[8,15], although some germination inhibitors (Huang et al., 1994; Huang et al., 1995c)[7,10], seed physiology (Huang et al., 1996b)[9] and hastening methods for germination (Huang et al., 1995a; Huang et al., 1996a)[5,4] were reported.

Therefore, in order to understand the dormancy mechanisms of American ginseng seed, the germination inhibitory effects of pulp were reported in the present paper. Subsequently, 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 will be discussed in these serial papers in succession.

MATERIALS AND METHODS

Plant material

All of the seeds used in our experiments were harvested from four-years-old American ginseng plants in middle September, 1992 on Huafu Ginseng Farm of Jilin Agricultural University, Changchun, China. The assay plant, Chinese cabbage (Brassica chinensis Linne) seed, was from Department of Horticulture, Jilin Agricultural University, Changchun, China.

Preparation of pulp juice

The fruit (berry) was hand-harvested, mechanically depulped. The pulp was put on a 20 mesh screen for getting the juice. After that, the juice was diluted as 0 (original juice), 1, 2, 5, 10, 50 and 100 times for bioassay and the distilled water was used as control.

Inhibitory effect bioassay of pulp juice

Pulp juice (3*ml*) of every concentration was put in a ø 9*cm* culture dish where a ø 7*cm* filter paper was put on the bottom. And then, the seeds of Chinese cabbage were put in 45~50℃ warm water for 10 min with stirring. Twenty-five seeds of Chinese cabbage were put in each culture dish and cultured in an incubator under the temperature of 28 ℃ for 48 hrs. At last, the radicle length of Chinese cabbage seed was measured. The experiments were performed three times as the same manner.

Treatment of depulping delay

Five different treatments of American ginseng fruit were treated as following:

CK : Depulping in 24 hrs after harvest,

A : Depulping delay for 15 days,

B : Depulping delay for 30 days,

C : Depulping delay for 60 days,

D : Never depulping during 270 days.

The seeds or fruit were mixed with mortar sand (1 vol seed or fruit / 4 vol sand) and the moisture content was adjusted to about 10%. The sand should be changed after depulping in every treatment. Stratification was conducted at the temperature of 20±1℃ for 270 days.

The lengths of embryo and endosperm were measured once every 15 days with a binocular microscope and the ER (Embryo ratio) was calculated by the following formula:

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

Inhibitory effects comparison among different pulp juice concentrations

The shorter was the radicle length of Chinese cabbage seed, the stronger was the inhibitory effect of American ginseng pulp juice. As can be seen in Fig. 1, the radicle length was 0 *mm* and the Chinese cabbage seed could not germinate at all in the original American ginseng pulp juice which showed a very strong inhibitory effect. When the juice was diluted to 1, 2, 5 and 10 times, the radicle lengths were 0.32±0.13, 0.72±0.20, 3.13±0.19 and 4.83±0.40*mm*, respectively and the inhibitory effects were the same level as that of original juice. Even when the juice was diluted to 20 and 50 times, the radicle lengths were 16.07±1.51 and 16.73±1.14 *mm*, respectively, which were still significantly stronger than that when juice was diluted to 100 time and CK with the radicle lengths of 23.50±4.65 and 25.98±2.93 *mm*, respectively. We know that there are some strong inhibitors in American ginseng pulp (Huang et al., 1995c)[10] and these inhibitors may be the main factor for inducing American ginseng seed dormancy by inhibiting the embryo growth. Therefore, when American ginseng seed is mature, the embryo is still not. We might deduce that when American ginseng seed developed on its mother plant, with the maturity of exocarp and midcarp, the inhibitors gradually accumulated in the pulp. While, when the inhibitors arrived to a certain quantity, they started to inhibit the growth of embryo. In this case, the embryo could not continue its developing until the pulp was removed after the fruit left its mother plant and the seed could not germinate until the embryo developed completely. That may be why the American ginseng seed needs quite a long time to pass through its morphological after ripening stage and break its dormancy. The results we got here were similar to those in Oriental ginseng seed (崔 高橋, 1977; 大禹 宮澤, 1958)[1,2].

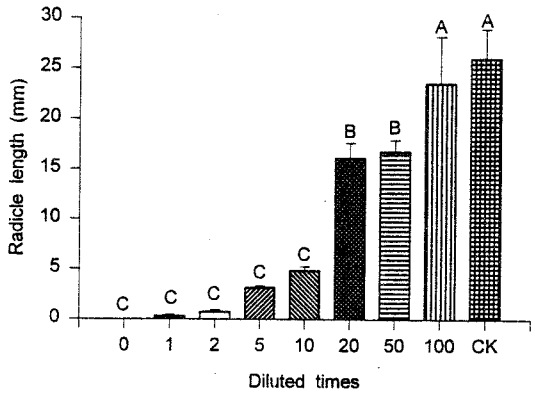

Fig. 1. Inhibitory effect comparison among different concentrations of American ginseng pulp juice tested by the radicle length of Chinese cabbage and the distilled water was used as control.
F-test and LSR were used (n=25, three replications).
Values that corresponded by the same letters were not significantly different at 0.01 level.

Effects of depulping delay on embryo growth

Our experiments here showed that the depulping delay seriously inhibited the growth of American ginseng embryo.

On the 15th day after treatment, the ER in CK (7.77%) was not significantly different with that in each treatment (7.69, 7.76, 7.19 and 7.20% in Treatment A, B, C and D, respectively) (Fig. 2, a). This showed us that although the pulp in CK was removed 15 days earlier than that in Treatment A, B, C and D, the residual inhibitory effect exerted from pulp had still not disappeared yet.

From the 30th to 75th day (Fig. 2, b, c, d and e), the ER in CK was significantly larger than that in all of other treatments (The ER in CK, A, B, C and D were 8.94, 7.54, 7.47, 7.14 and 7.32% on the 30th day; 12.09, 8.09, 7.70, 7.51 and 7.49% on the 45th day; 16.58, 9.73, 7.45, 6.43 and 6.43% on the 60th day and 19.55, 13.42, 7.62, 6.92 and 6.54% on the 75th day, respectively). We could realize that on the 30th day after depulping, the residual inhibitory effect from pulp disappeared in CK. However, Treatment A (depulping delay for 15 days), B (depulping delay for 30 days) and C (depulping delay for 60 days) were depulped successively in this period,

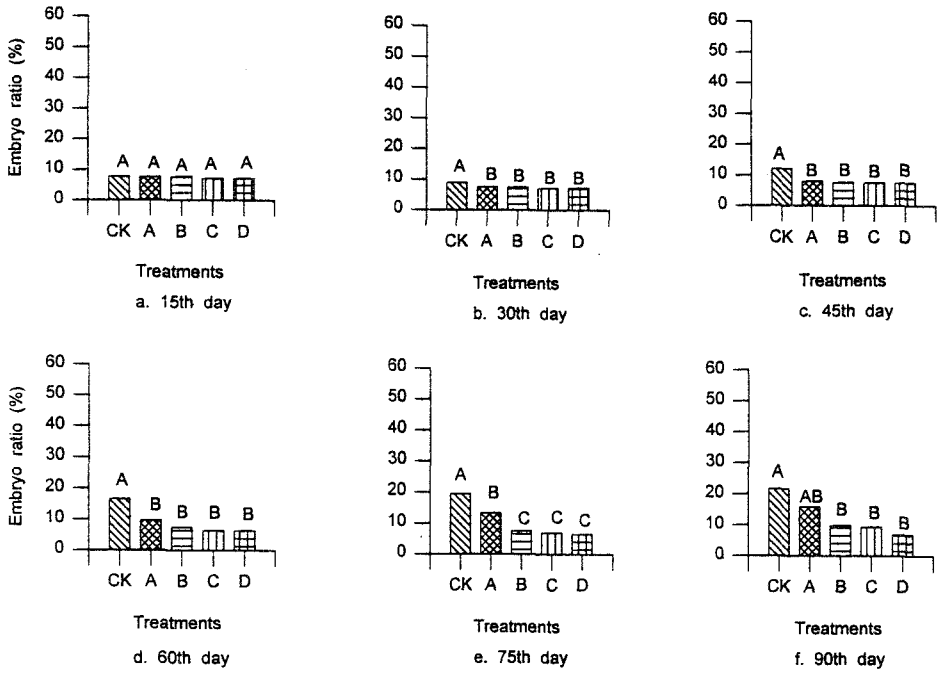

Fig. 2 a-f. Influence of depulping delay on the growth of American ginseng embryo.
CK=Depuling in 24 hrs after harvest,
A=Depulping delay for 15 dats,
B=Depulping delay for 30 dats,
C=Depulping delay for 60 dats,
D=Depulping delay for 270 dats.
Values that corresponded by the same letters were not significantly different at 0.01 level.

but the residual inhibitory effect from pulp had not disappeared for some periods.

From the 90th to 150th day (Fig. 2, f, g, h, i and j), the ER in Treatment A was not significantly different with that in CK (The ER in CK, A, B, C and D were 21.72, 15.93, 9.92, 9.44 and 7.00% on the 90th day; 21.06, 16.32, 10.22, 9.53 and 8.67% on the 105th day; 23.42, 21.64, 13.63, 12.20 and 9.98% on the 120th day; 27.04, 27.56, 12.45, 8.92 and 7.93% on the 135th day and 27.70, 23.08, 14.65, 11.42 and 12.08% on the 150th day, respectively). This indicated that the pulp was removed on the 15th day after harvest, but the residual inhibitory effect from pulp disappeared until the 90th day. On the other word, in needs 75 days for extricating the residual inhibitory effect from pulp.

From the 165th to 180th day (Fig. 2, k and l), Treatment A and B were not significantly different with CK (The ER in CK, A, B, C and D were 29.26, 27.35, 24.73, 14.90 and 10.58% on the 165th day and 30.26, 24.08, 18.48, 12.55 and 9.44% on the 180th day, respectively). This made us understand that the pulp was removed on the 30th day and the residual inhibitory effect disappeared on the 165th day. Therefore, 135 days were needed.

After the 195th day, the ER in Treatment C

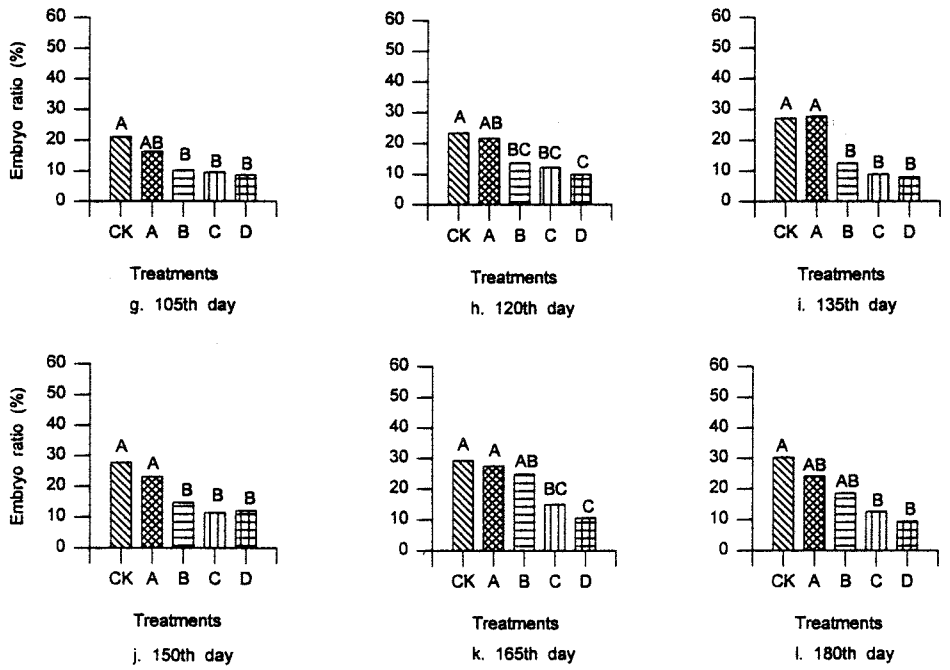

Fig. 2 g-l. Influence of depulping delay on the growth of American ginseng embryo.
CK=Depuling in 24 hrs after harvest,
A=Depulping delay for 15 dats,
B=Depulping delay for 30 dats,
C=Depulping delay for 60 dats,
D=Depulping delay for 270 dats.
Values that corresponded by the same letters were not significantly different at 0.01 level.

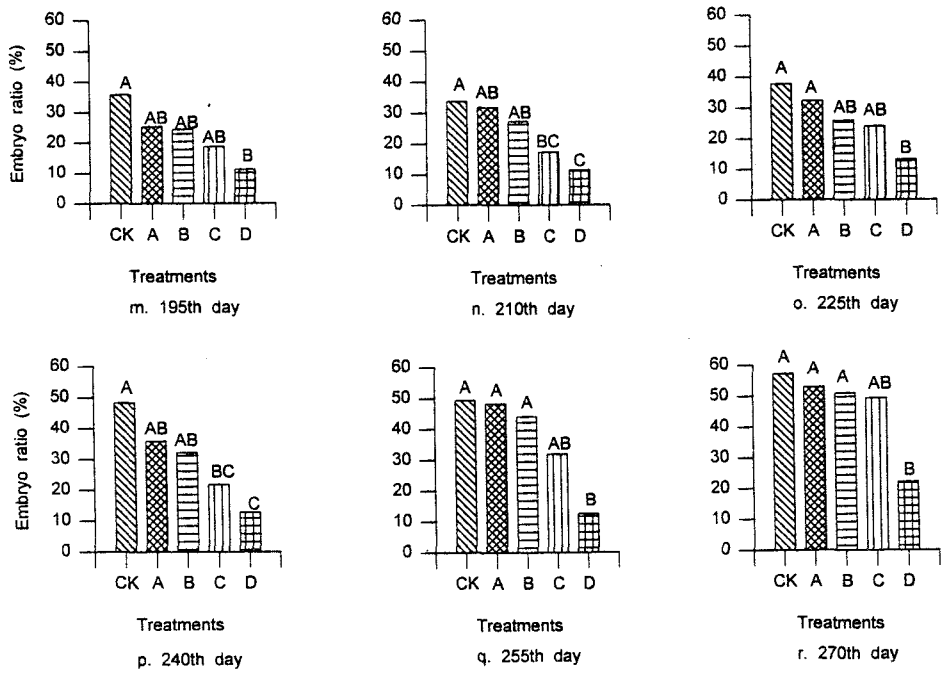

Fig. 2 m-r. Influence of depulping delay on the growth of American ginseng embryo.
CK=Depuling in 24 hrs after harvest,
A=Depulping delay for 15 dats,
B=Depulping delay for 30 dats,
C=Depulping delay for 60 dats,
D=Depulping delay for 270 dats.
Values that corresponded by the same letters were not significantly different at 0. 01 level.

gradually arrived to the same level as that in CK (The ER in CK, A, B, C and D were 35. 77, 25. 24, 24. 14, 18. 53 and 11. 17% on the 195th day; 33. 74, 31. 85, 27. 24, 17. 25 and 11. 35% on the 210th day; 37. 67, 32. 29, 25. 82, 24. 04 and 13. 22% on the 225th day; 48. 38, 35. 69, 32. 21, 21. 89 and 12. 81% on the 240th day; 49. 34, 48. 30, 44. 03, 31. 92 and 12. 56% on the 255th day and 57. 30, 53. 05, 51. 03, 49. 37 and 22. 18% on the 270th days, respectively), it needed 135 days for the seed removed pulp on the 60th day to extricate the residual inhibitory effect (Fig. 2, m, n, o, p, q and r).

However, the ER in Treatment D (never depulping) was always significantly different with that in CK. This pointed that the inhibitory effect from pulp never disappeared from the beginning until the 270th day although the pulp rotted off much early before the 270th day. The duration for extricating the residual inhibitory effect from pulp in CK, Treatment A, B and C were 30, 75, 135 and 135 days, respectively. The longer was the duration that the pulp stayed around the seed after harvest, the longer was the time course needed for embryo getting free from the inhibitory effects of pulp after depulping.

Effects of depulping delay on seed rotten ratio

As can be seen in Fig. 3, the seed rotten ratios in CK, Treatment A, B, C and D were 5. 00, 4. 17, 17. 50, 19. 17 and 20. 83% on the 120th day, 4. 49,

5.13, 18.59, 24.36 and 27.56% on the 180th day and 5.47, 5.71, 19.05, 27.14 and 33.33% on the 270th day, respectively. This experiment showed that the seed rotten ratios in Treatment B, C and D were much higher than those in CK and Treatment A. The longer was the duration that pulp stayed around the seed after harvest, the higher was the rotten ratio of American ginseng seed.

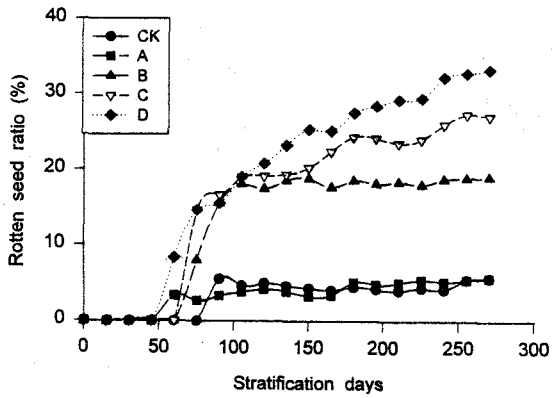

Fig. 3. Influence of depulping delay on the rotten ratio of American ginseng seed.
CK=Depuling in 24 hrs after harvest,
A=Depulping delay for 15 days,
B=Depulping delay for 30 days,
C=Depulping delay for 60 days,
D=Never depulping during 270 days.

All of the experiments reported here demonstrated that pulp was one of the main inhibitory factors which made American ginseng seed get into dormancy. Particularly, some inhibitors in pulp might inhibit the embryo morphological afterripening. The longer was the duration that pulp stayed around the seed, the stronger was the inhibition that the pulp inhibited embryo growth, the higher was the seed rotten ratio and the larger was the pecuniary loss for us. For this reason, we should depulp immediately after the harvest of American ginseng fruit and wash the seed with water repeatedly until the seed with a light color to get rid of the inhibitory effects from pulp as early as possible.

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摘 要

본연구는 西洋蔘(*Panax quinquefolium* Linne) 果肉에 의한 Brassica(*Brassica chinensis* Linne) seed를 대상으로 發芽 억제작용을 검토하였다. 서양삼果汁에는 發芽억제물질이 존재하고, 그 억제작용은 희석도에따라 감소하였다. 즉 희석배수가 0, 1, 2, 5, 10, 20, 50, 100일때 胚根의 길이는 각각 0, 0.32, 0.72, 3.13, 483, 16.07, 16,73, 23.50 mm이였다(CK=25.98 mm). 과육의 존재는 種胚의 성장을 현저하게 억제하였다. 收穫후 과육이 종자 표면에 부착한 시간이 0, 15, 30, 60일 때 종자 생장에 대한 억제 시간은 각각 30, 75, 135, 135일로 나타났다. 종자 썩는 비율도 높아졌다. 과육의 부착한 시간이 0, 15, 30, 60, 270일 때 종자 썩는 비율은 각각 5.47, 5.71, 19.05, 27.14, 33.33%로 나타났다. 이러한 결과는 과육에 西洋蔘種子休眠을 일으키는 주요한 억제성분이함유되어 있다는 것을 시사하였다.

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