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Study on Dormancy Mechanism and Breaking Dormancy Method of *Viburnum sargentii* Seeds

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Abstract

Under untreated conditions, the germination rate of *Viburnum sargentii* seeds is very low. By exploring the relationship between the dynamic changes of physicochemical indicators and endogenous hormones and seed germination during the dormancy and germination of *Viburnum sargentii* seeds, the mechanism of seed germination of *Viburnum sargentii* was determined, which provided a theoretical basis for its extensive promotion and development. The contents of soluble sugar and soluble starch were determined by anthrone colorimetry. The soluble protein was determined by Coomassie brilliant blue G-250 staining. The activity of polyphenol oxidase (PPO) was determined by NBT reduction method. The peroxidase (POD) was determined by guaiacol method. And the endogenous hormones ZA, IAA, ABA, GA_3 were determined by liquid chromatography. Results show: 1) Under natural conditions, the seeds of *Viburnum sargentii* are difficult to germinate. 2) Under sand storage for 8 months, the germination rate of untreated seeds was 33%. Puncture and peeling treatment could significantly increase the germination rate, and the germination rate of seeds treated with puncture was 92%, and that treated with peel was 98%. 3) Seed germination was accompanied by the decrease in macromolecular substances such as soluble sugar, soluble starch, and soluble protein. 4) The dynamic changes of hormones during seed germination conform to the hypothesis of "three factors". 5) The treatment of puncture and peeling increased the content of endogenous hormones promoting germination, decreased the endogenous hormones inhibiting seed germination, and increased the ratio of (IAA + GA + ZR)/ABA or GA/ABA. The seeds of *Viburnum sargentii* have obvious dormancy characteristics. Under the condition of sand storage, both pricking and peeling treatment can effectively promote the process of breaking dormancy, and the effect of peeling treatment is better.

Keywords

Viburnum sargentii, Dormancy Mechanism, Endogenous Hormones, Breaking Dormancy

1. Introduction

Viburnum sargentii belongs to *Caprifoliaceae*, *Viburnum*, shrub, 2 - 3 m high, umbelacrogenesis. Its crown, leaf shape, flower posture and fruit are very beautiful. In summer, we can see the infertile radiation flowers of its outer periphery. In autumn, we can see its bright red fruits. In a certain planting environment, some leaves are red in autumn, and there are few pests and diseases. It is an excellent flower and shrub species in gardens [1]. Due to the dormancy of *Viburnum sargentii* seeds, the wide spread promotion of *Viburnum sargentii* is limited. However, the research on the dormancy mechanism of the seeds of *Viburnum sargentii* is still rare at home and abroad, only Hu Yongqiang [2] studied the breeding experiment of *Viburnum sargentii*. The results showed that the seeds were successfully planted in spring after treating with variable temperature sand. This may be related to the fact that sand storage promotes seed to break dormancy. At present, under the conventional sowing condition, the germination rate of *Viburnum sargentii* is very low, and the research on dormancy mechanism of *Viburnum sargentii* seeds at home and abroad is still blank, which affects the promotion and utilization of *Viburnum sargentii*. Therefore, the physiological property and endogenous hormone levels of *Viburnum sargentii* seed were studied in this study in order to solve the problem of seed dormancy and this study provided a method to effectively shorten the dormancy time and provided a theoretical basis for the wide promotion and development and utilization of *Viburnum sargentii*.

2. Materials and Methods

2.1. Overview of the Experimental Field

The experimental field is located in the forestry experimental base of Shandong Agricultural University. It is located in the southeastern part of Taishan District, Tai'an City. It has a warm temperate semi-humid continental monsoon climate with four distinct seasons. The annual average temperature is 13.2 C, the annual average precipitation is 803.7 mm, the annual average sunshine is 2627.1 h, the soil is loam, and the fertility is moderate.

2.2. Experimental Materials

The materials used in the experiment were collected from the experimental field of the Southern Campus of Shandong Agricultural University in mid-October 2016. After harvesting, the fruits were chopped in net bags, and rinsed several times, and the pulp, peel and shriveled seeds were removed. After fishing out, the ventilated place was dried and the selection was full. The seeds are dried and ready for use. The average seed weight of the tested seeds was 49.12 g.

2.3. Experimental Methods

In this experiment, three differently treated seeds (untreated, pricked seed coat, peeled seed coat) were stratified germination, and the sand and seeds were mixed in a ratio of 3:1 (the water content of sand used for stratification is kept at about 60%), and then put it into the seed collection bag, burring at a depth of about 40 cm and then adding sand. The sand storage is started from April, and the seeds are taken once every 30 days to determine the germination rate, physical and chemical properties and endogenous hormone changes of the seeds. It lasted 8 months for 240 days.

2.3.1. Observation of the Structure and Morphology of Seeds

Three sets of the above-mentioned spare seeds were randomly selected, 100 pieces in each group, soaked in clear water for 2 days, so that the seeds were fully absorbed to remove the seed coat (endocarp), and then the whole embryo was peeled off with a scalpel, and the embryos were laterally and longitudinal anatomy in order to observe the internal morphological structure of the seed, and record.

2.3.2. Determination of Seed Physical and Chemical Indicators

The contents of soluble sugar and soluble starch were determined by anthrone colorimetry [3]. The soluble protein was determined by Coomassie brilliant blue G-250 staining. The activity of polyphenol oxidase (PPO) was determined by NBT reduction method [4] [5], enzyme activity unit (U) was expressed as an increase of 0.01 per minute (ΔA460) per gram of sample. The peroxidase (POD) was determined by guaiacol method [6], enzyme activity unit (U) was increased by 0.01 per minute per gram of fresh sample (ΔA460).

2.3.3. Determination of Endogenous Hormones in Seeds

The endogenous hormones zeatin (ZA), auxin (IAA), abscisic acid (ABA), gibberellin (GA_3) were determined by liquid chromatography [7].

3. Results and Analysis

3.1. External Morphological Characteristics of the Seeds of *Viburnum sargentii*

The fruit of *Viburnum sargentii* is a drupe with nearly round seeds. The longitudinal axis of the seed is 6.1 - 7.3 mm, the horizontal axis is 5.4 - 5.9 mm, the thickness is 1.4 - 1.9 mm, the base is round, the apex is gradually narrow, and the surface is taupe. The seed coat is thick and hard.

Among the 100 seeds dissected, the embryos were 100% complete, with plumule, hypocotyls and radicles. Without endosperm, the embryo is milky white, and the two cotyledons are thick and full of embryonic cavity.

3.2. Seed Germination Rate under Different Treatment

During the sand storage process lasting 8 months (indicated by the numbers 1 - 8 in the table below), the difference of seed germination performance among different treatments was large. The germination rate of the untreated seed stored in sand for 8 months was only 33% (Figure 1). The germination rate of the punctured and peeled seeds was significantly higher than that of the untreated seeds, reaching 92% and 98%, respectively. Analysis of variance (Table 1) showed that the germination rate of seeds treated by puncture and peeling was extremely significant (P < 0.01).

3.3. Effects of Different Treatments on the Physicochemical Properties of Seeds

3.3.1. Analysis on the Changes of Soluble Sugar Content under Different Treatments

The change of soluble sugar content in the seeds of *Viburnum sargentii* showed a decreasing trend during the process of sand storage (Figure 2). Before the sand storage, the soluble sugar contents in the seeds of three different treatments were 852.53 μg/g, 647.32 μg/g, and 743.82 μg/g (in the order of untreated, punctured, peeled, followed by the same). After 4 month of sand storage, the lowest values were obtained, which were 184.19 μg/g, 299.3 μg/g, and 384.16 μg/g, respectively. The change of solube sugar content in the seeds showed a increasing trend during 4 - 5 months of sand storage. At 5 month of sand storage, the maximum

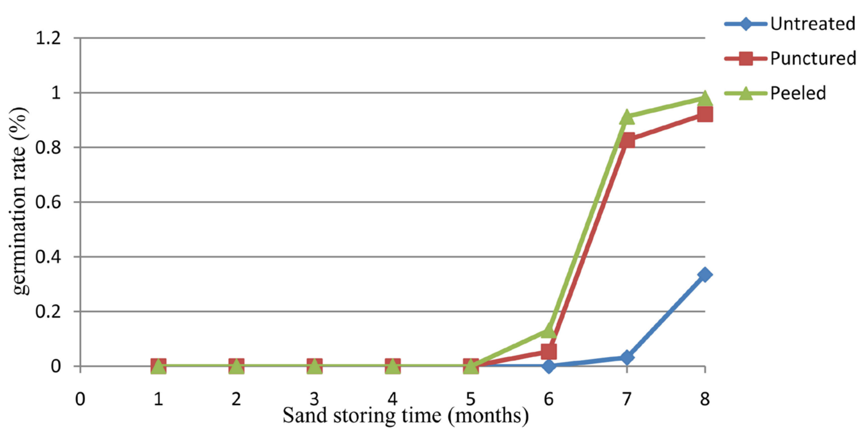

Figure 1. Variation of seeds germination rate of *Viburnum sargentii* with time under different treatments.

Table 1. Seeds germination rate of *Viburnum sargentii* under different treatment*.

Sand storing time	Untreated (%)	Punctured (%)	Peeled (%)
1 - 5 th	0	0	0
6th	0C	5.4 ± 0.9B	13.2 ± 1A
7th	3. 2 ± 1.2C	82.7 ± 4.2B	91.3 ± 2.9A
8th	33.4 ± 2.8C	92.2 ± 2.1B	98.1 ± 1.7A

*Different capital letters on the same line show significant differences.

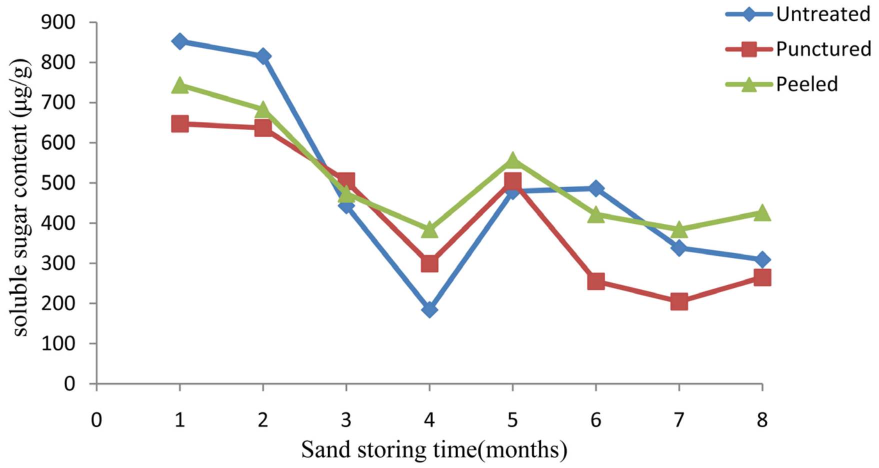

Figure 2. Variation of soluble sugar in seeds of *Viburnum sargentii* under different treatments.

values reached 478.9 μg/g, 504.68 μg/g, 557.14 μg/g, respectively, and then began to decrease. The content of soluble sugar in the untreated seeds decreased to 309.15 μg/g at 8 month. The content of soluble sugar in the punctured and peeled seeds began to increase after 7 month of sand storage (204.8 μg/g, 384.15.8 μg/g), and the content of soluble sugar was 265.11 μg/g and 426.31 μg/g at 8 month of sand storage.

3.3.2. Analysis on the Changes of Soluble Starch Content under Different Treatments

The change of soluble starch content in the seeds of *Viburnum sargentii* showed a decreasing trend during the process of sand storage and it increased slightly during the reduction process (**Figure 3**). The content of soluble starch in the untreated seeds was 181.91 mg/g before sand storage, and decreased to 79.59 mg/gat 4 month of sand storage. The soluble starch content showed an increased trend at 4 - 5 months of sand storage. At 5 month of sand storage, the maximum value reached 105.56 mg/g, and then lasted decrease. At 8 month, the lowest value reached 30.64 mg/g. The content of soluble starch in the punctured and peeled seeds were 125.16 mg/g and 143.26 mg/g, respectively, before sand storage. At 3 months of sand storage, the maximum value reached 129.36 mg/g and 116.5 mg/g, respectively, and then lasted decrease. The soluble starch content of the punctured seeds reached a minimum value of 67.2 mg/g at 5 months, and increased to 97.68 mg/g at 8 months. The soluble starch content of the peeled seeds reached a minimum value of 57.14 mg/g at 7 months, and increased to 79.25 mg/g at 8 months.

3.3.3. Analysis on the Changes of Soluble Protein Content under Different Treatments

Under different treatments, the change of soluble protein content in the sand storage process is shown in **Figure 4**. As the sand storage time prolongs, the soluble protein content shows a continuous decreasing trend. The content of

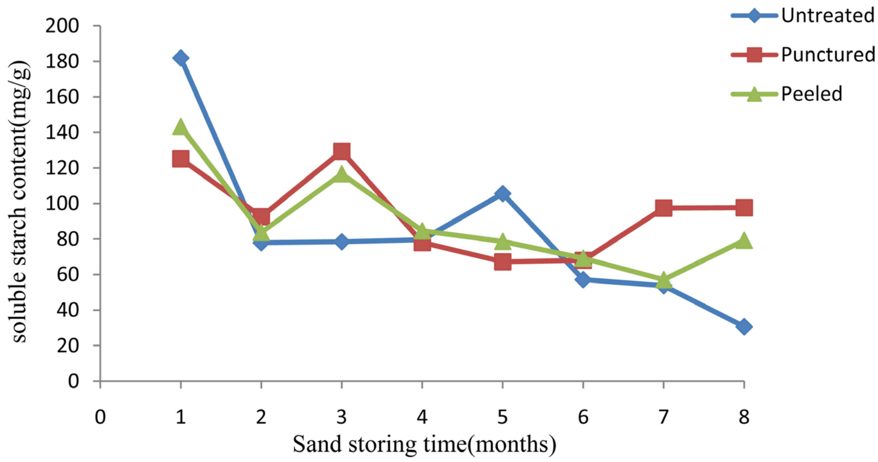

Figure 3. Variation of soluble starch in seeds of *Viburnum sargentii* under different treatments.

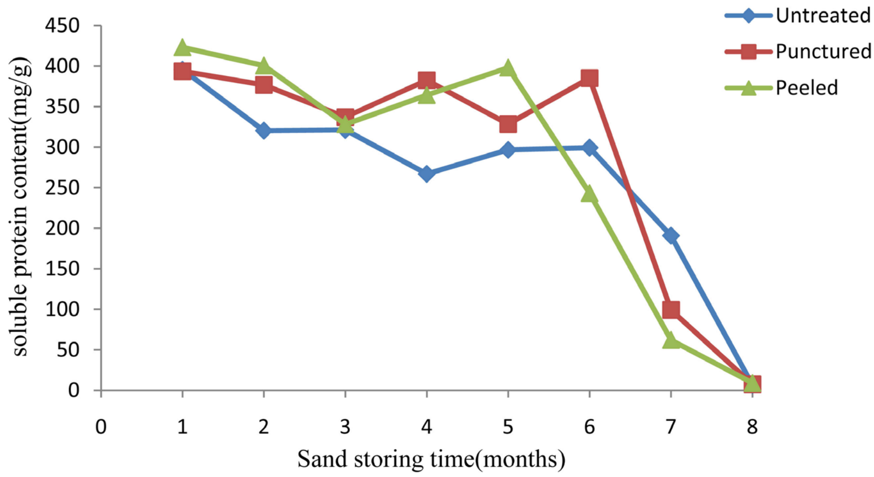

Figure 4. Variation of soluble protein in seeds of *Viburnum sargentii* under different treatments.

soluble protein in the seeds was about 400 mg/g before sand storage, and about 300 mg/g at 5 month. The amount of change was not large. The soluble protein content decreased significantly after 5 months, and the lowest value was about 10 mg/g at 8 months.

3.3.4. Analysis on the Changes of Peroxidase POD Activity under Different Treatments

The change of POD activity in seeds during the process of sand storage showed a trend of decrease-rise-reduction (**Figure 5**). The endogenous POD activity of the untreated seeds was 10 U before sand storage, 3 U at 6 months and then increased rapidly. The activity reached a maximum of 40 U at 7 months, and then decreased to 4 U at 8 months. The endogenous POD activity of punctured and peeled seeds was similar. The POD activity before sand storage was 14 U and 19 U, respectively, reaching the maximum of 54 U and 46 U at 4 months, and 10 U and 14 U at 5 months, respectively. The subsequent changes were not significant.

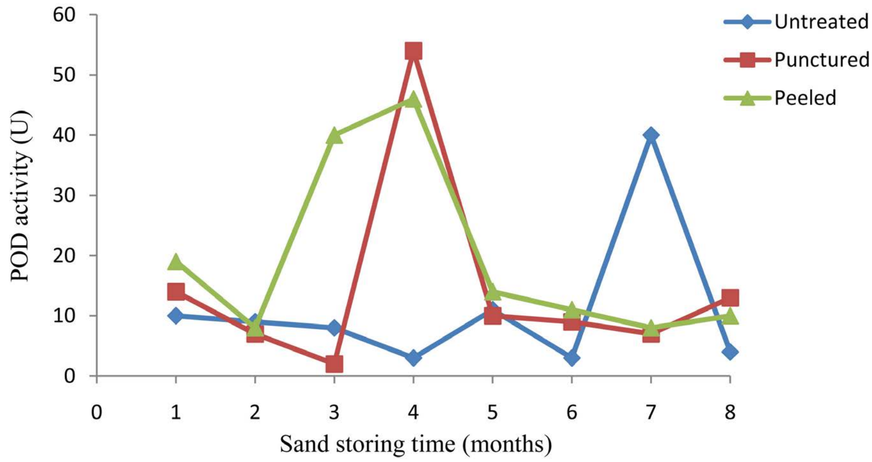

Figure 5. Variation of POD activity in seeds of *Viburnum sargentii* under different treatments.

3.3.5. Analysis on the Changes of Polyphenol Oxidase PPO Activity under Different Treatments

The change of PPO activity in seeds during the process of sand storage showed a trend of decrease-rise-reduction (**Figure 6**). The endogenous PPO activity of the untreated seeds was 1.22 U before sand storage, and decreased to 0.29 U at 2 months. It showed not much change during 2 - 6 months, and increased to the maximum value of 1.29 U at 7 months. The minimum value was 0.035 U at 8 months. The endogenous PPO activity of punctured and peeled seeds was similar. The PPO activity were 1.7 U and 0.85 U before sand storage, respectively, decreased to 0.11 U and 0.06 U at 2 months, and then began to rise slowly. At 5 months, the enzyme activity reached a maximum of 0.41 U and 0.27 U, respectively, and began to decline after 5 months, reaching a minimum of 0.045 U and 0.062 U at 7 months. A slight increase at 8 months was 0.165 U and 0.117 U, respectively.

3.4. Effects of Different Treatments on Changes of Endogenous Hormones in Seeds

3.4.1. Analysis on the Changes of Endogenous ZA Content with Time under Different Treatments

With the sand storage prolonged, the ZA content in the seeds showed an overall upward trend (**Figure 7**). The content of endogenous ZA in the three treatment seeds before sand storage was 0.15 μg/g, 0.15 μg/g, 0.21 μg/g, respectively. In the process of sand storage, the phenomenon of small fluctuations showed an overall increase. At 8 months, the content of endogenous ZA was 0.39 μg/g, 0.31 μg/g, 0.55 μg/g.

3.4.2. Analysis on the Changes of Endogenous IAA Content with Time under Different Treatments

The content of IAA showed a slow rise-rapid decline trend (**Figure 8**). The content of IAA was 0.39 μg/g, 042 μg/g and 0.34 μg/g respectively before sand storage. The IAA content increased continuously from 1 to 7 months, and reached

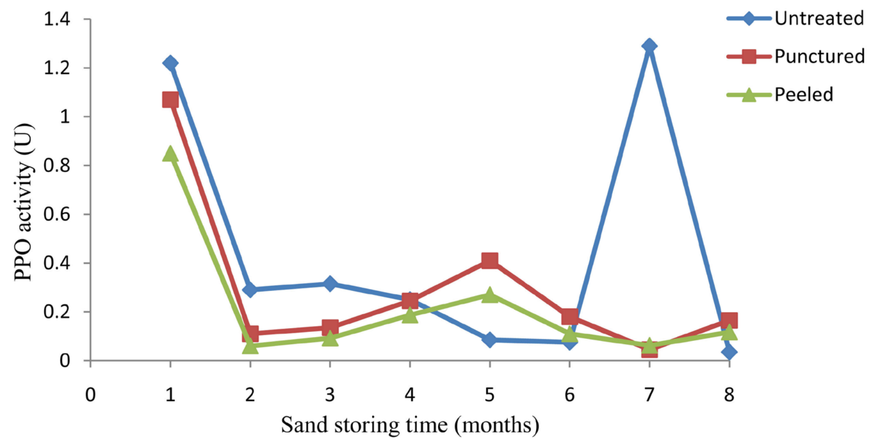

Figure 6. Variation of PPO activity in seeds of *Viburnum sargentii* under different treatments.

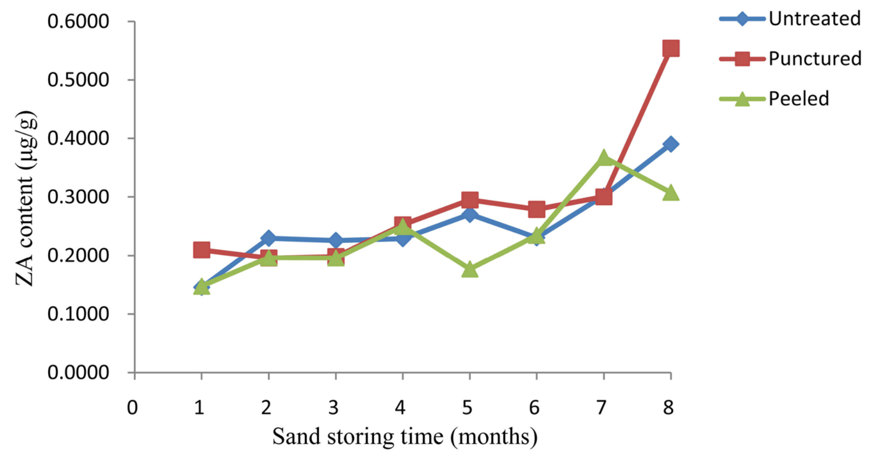

Figure 7. Variation of endogenous ZA in seeds of *Viburnum sargentii*.

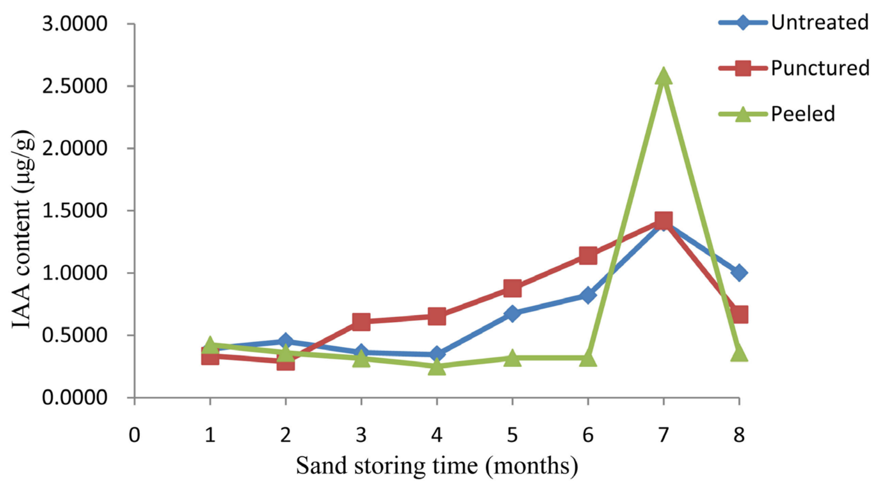

Figure 8. Variation of endogenous IAA in seeds of *Viburnum sargentii*.

the maximum value of 1.41 μg/g, 2.59 μg/g, 1.42 μg/g at 7 months. The IAA content decreased to 1 μg/g, 0.36 μg/g, 0.67 μg/g, respectively, at 8 months.

3.4.3. Analysis on the Changes of Endogenous ABA Content with Time under Different Treatments

The content of ABA showed a trend of decreased-flat-upward (**Figure 9**). The content of ABA were1.21 μg/g, 1.52 μg/g and 1.8 μg/g, respectively before sand storage, and decreased to 0.61 μg/g, 0.62 μg/g, 0.53 μg/g at 2 months. There was not much change during 2 to 6 months. It began to increase at 7 months, and reached 1 μg/g, 0.86 μg/g, 1.3 μg/g at 8 months.

3.4.4. Analysis on the Changes of Endogenous GA Content with Time under Different Treatments

The content of endogenous hormone GA in untreated seeds showed a rising trend (**Figure 10**), which was 0.20 μg/g before sand storage and 1.13 μg/g at 8 months. The GA content in the seeds of puncture and peeling treatment increased significantly and then decreased. The content were 0.09 μg/g and 0.46 μg/g before sand storage, and increased to the maximum value of 2.09 μg/g and 1.61 μg/g at 4 months, and then decreased. The content of GA were 0.23 μg/g and 0.33 μg/g, and rooting occurred. The GA in the untreated seeds continued to show an elevated process, and the concentration was low. A large number of rooting occurred at 8 months.

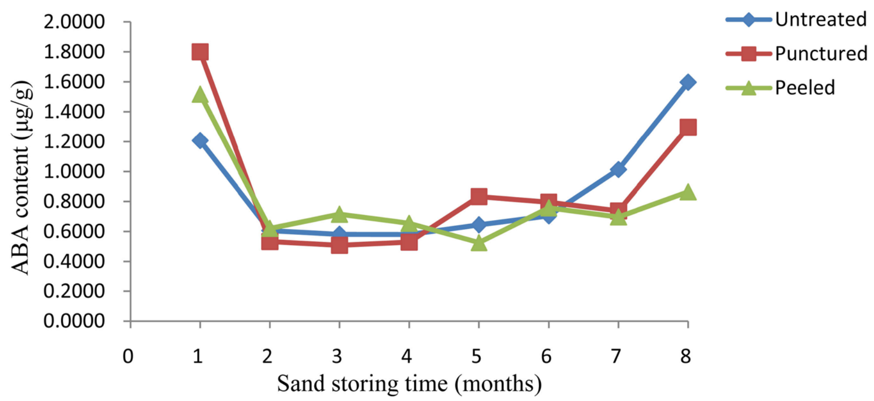

Figure 9. Variation of endogenous ABA in seeds of *Viburnum sargentii*.

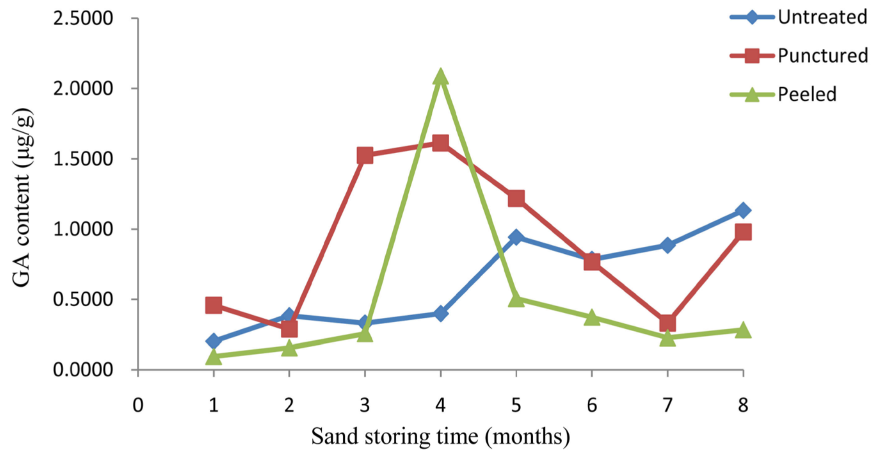

Figure 10. Variation of endogenous GA in seeds of *Viburnum sargentii*.

3.4.5. Analysis of Synergistic Effects of Hormones in Seeds of Each Treatment

Under the three different treatments, the ratio of endogenous hormone (IAA + GA + ZR)/ABA in the seed showed a trend of increase-decrease-increase-decrease (Figure 11), which led to the development of seeds in the direction of germination, and the seeds of puncture and peeling treatment reached the peak value ratio reached 3.2 or more, indicating that the hormone accumulation for seed germination was larger, and the seed endogenous hormone (IAA + GA + ZR)/ABA peaks were more than one month earlier than the punctured seeds. The untreated seed reached the first peak with a ratio of 1.75, and the second peak had a ratio of 2.93. The ratio was small, indicating that the hormone accumulation for seed germination was less, and the overall ratio was lower than the two treated seeds.

4. Discussion

4.1. Physiological and Biochemical Changes of Seeds during Sand Storage

During the process of seed breaking dormancy, the storage substances such as starch, fat, protein and other organic substances have undergone a series of metabolic transformation processes such as hydrolysis, transportation and reconstruction. La Croix believes that seeds are metabolized during the post-ripening period to produce soluble sugars and are used for respiration, macromolecular proteins are also broken down to produce soluble proteins, and proteolytic enzyme activity is also increased [8]. The physiological and biochemical changes of seeds treated by sand storage can be found that the germination of seeds is accompanied by the degradation of macromolecular substances such as soluble sugar, soluble starch and soluble protein, indicating that the process of seed from dormancy to germination is the process of continuously consuming energy. In view of changes in biological enzyme activity, POD generally has higher activity in aging tissues, and the activity in young tissues is weaker, and it tends

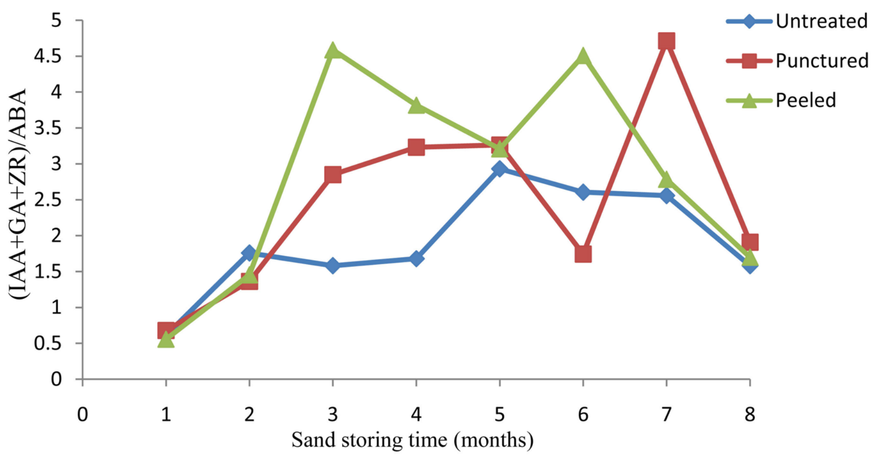

Figure 11. Change in the ratio of endogenous hormones in seeds of *Viburnum sargentii*.

to increase first and then decrease in dormant seeds, and the seeds for peeling treatment start first, followed by puncture. The untreated seeds have the slowest change. Therefore, it can be speculated that the seed germination of *Viburnum sargentii* needs to undergo an aging process, and the peeling and puncture treatments promote the process to varying degrees; the phenolic substances are some seeds' inhibitor of germination. The PPO activity of hawthorn seed was significantly increased after sand storage, which was beneficial to the oxidation of phenolic substances, which was consistent with the decrease of phenolic content in seeds after sand storage [9]. In the process of sand storage, the PPO activity in different treated seeds increased significantly, and the PPO activity in the seeds of puncture treatment and peeling treatment increased earlier than untreated seeds for two months. The phenolic content in the puncture and peeling seeds decreased earlier, which was consistent with the earlier rooting of the treated seeds. The PPO content in the untreated seeds reached the maximum at 7 months of sand storage. It indicated that the phenolic substances accumulated in the untreated seeds were more accumulated and the decomposition was slower, which was consistent with the lowest germination rate in the sand for eight months.

4.2. Dynamic Changes of Endogenous Hormones in the Process of Breaking the Dormancy of *Viburnum sargentii* Seeds

The regulation mechanism of seed germination and dormancy is still inconclusive. The most accepted is the "plant hormone three-factor" hypothesis proposed by Kham and Waters. During the process of sand storage in *Viburnum sargentii* seeds, the ZA content of different treatments showed a gradual increase trend, and the concentration did not change much, indicating that ZA did not play a leading role in seed germination. IAA content continued to rise during sand storage, but decreased at 7 months. It is speculated that it may be related to the inhibition of ABA and the promotion of GA. The content of GA in the seeds of the three treatments reached a high level before germination, which was consistent with the "three factors hypothesis".

During the germination of seeds, a variety of hormones play a regulatory role. Different treatments of seeds can affect changes in endogenous hormones in the seed, which in turn affect seed germination. Previous studies have found that seed germination is accompanied by the disruption of endogenous hormone balance, the contents of GA, IAA and ZA that promote germination are increased, and the contents of IAA that inhibit germination are decreased. The results showed that the contents of endogenous hormones GA and IAA in seeds treated with puncture and peeling were higher than those in untreated seeds, while ABA content was the opposite. Puncture and peeling treatment increased the content of endogenous hormones promoting germination, decreased the endogenous hormones inhibiting seed germination, and broke the balance of endogenous hormones in seeds, (IAA + GA + ZR)/ABA or GA/ABA ratio increased, and developed towards seed germination. This is basically consistent with previous studies on seed germination of *Tilia amurensis* [10], *Cyclocarya paliurus* [11], and *Idesia polycarpa* [12].

Under the conditions of sand storage, puncture and peeling treatment can promote the dormancy of *Viburnum sargentii* seeds. The main manifestation is that the increase of endogenous hormone (IAA + GA + ZR)/ABA ratio will promote seed germination, both treatments shortened the time when the ratio reached its peak value. When the seeds of the punctured treatment reached the peak of 4 months and 7 months respectively, the two peaks of the peeled seeds were one month earlier than the seeds of the punctured treatment. It is presumed that the seeds reached the initial stage when the first peak was reached. At the stage, the embryo begins to sprout, and when the second time reaches the peak, new shoots appear, which coincides with the germination time and germination rate of the corresponding treated seeds. The untreated seeds peak at 2 months and 5 months, and the first peak ratio is lower, the hormone accumulation for seed germination is not high. So it enters the early stage of germination later, the germination time is later than the puncture and peeling treatment, the germination rate is lower than the two treatments, mainly with the germination time and the hormone accumulation that promotes seed germination. The slower growth has a major relationship. This is the same as the previous studies on Styraxobassia [13] and Ferula fukanensis [14].

After puncture and peeling treatment, sand storage can effectively shorten the speed of breaking dormancy of *Viburnum sargentii* seeds and increase the germination rate. According to the dynamic changes of seed physiological property and endogenous hormone content in different periods, treating seeds with GA during 4 months of sand storage may promote faster breaking of dormancy. This is consistent with previous studies on the effect of Gibberellin on seed germination of *Solanum indicum* L. [15], *Tribulus terrestris* L. [16].

5. Conclusion

Under natural conditions, the seeds of *Viburnum sargentii* are difficult to germinate. Under the condition of 8 months of sand storage, the germination rate of untreated seeds is 33%, and the germination rate can be significantly increased by puncture and peeling treatment, and the germination rate of the seeds treated by puncture is 92%, the germination rate of peeling treatment is 98%. Puncture and peeling treatment could significantly increase the content of endogenous hormones IAA and GA, which promoted seed germination, and reduced the content of endogenous hormone ABA, which inhibited germination, so that (IAA + GA + ZR)/ABA ratio is increased, which in turn promotes seed germination. Puncture and peeling treatment are beneficial to the seed breaking dormancy, and the peeling treatment can enter the seed germination stage earlier and the germination rate is higher.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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