Int. j. adv. multidisc. res. stud. 2023; 3(2):416-419

Received: 02-02-2023
Accepted: 11-03-2023

International Journal of Advanced Multidisciplinary Research and Studies

ISSN: 2583-049X

Panax Vietnamensis var. *Vietnamensis* Germination Using Specific Seed Chip

[1] Nguyen Thi Phuong Trang, [2] Nguyen Quang Thach

[1] Institute of Ecology and Biological Resource, Vietnam Academy of Science and Technology (VAST), Hanoi, Vietnam

[1] Graduate University of Science and Technology (GUST), Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam

[2] Vietnam National University of Agriculture, Hanoi, Vietnam

Corresponding Author: **Nguyen Thi Phuong Trang**

Abstract

Panax vietnamensis var. *vietnamensis* Ha et Grushv. has been identified as a precious medicinal plant of Vietnam, ranked as one of the four most valuable ginseng in the world because of its saponin, amino acid, minerals…are much better than other ginseng. Professional agencies have been appreciated very high them in economic value, usefulness in treatment, health improvement…but they are now absent in natural environment. Because of limited distribution and over exploitation, *Panax vietnamensis* var. *vietnamensis* Ha et Grushv. fell into the threatened group. The greatly expansion of plantation size is also making scarcity of seedlings the more day, the more pressing. Vietnam ginseng tissue cultivation has been successfully by several research institutes but the tissue cultivation plants have vitality not good as the plants grown from seed. Vietnam ginseng still in the state of supply is not enough demand, thus, the prices of ginseng products and ginseng seeds are still very high. This our research applied Seed chip technology of Korea for growing Ngoc Linh Ginseng from seed. Initial research results show that 100% of seeds grown on the seed chip are protected from the effects of mold, bacteria and rodents. 100% of seeds grown on the seedchip are germinated and developed into seedlings. There are 95,83% of Ngoc linh ginseng seedlings released into the natural environment grow well in the first 6 months.

Keywords: *Panax Vietnamensis* Var. *Vietnamensis*, Seedchip Germonation, Conservation of Genetic Resources

Introduction

Panax vietnamensis var. *vietnamensis* (Ngoc Linh ginseng) was defined as a precious medicinal plant of Vietnam, listed to one of the four most valuable ginseng species in the world because it contains the quantity of saponins, amino acid and mineral much more than other ginseng species, was high evaluated on economic value, on health improvement and healing by specialized organizations but it was absented in the natural environment. Moreover, due to limited distribution and overexploited made *Panax vietnamensis* var. *vietnamensis* fall into the threatened group [5]. At presente, this species is only cultivated in several conservation places in Quang Nam and Kon Tum provinces. *Panax vietnamensis* var. *vietnamensis* has been put into red list of IUCN [10]. The health ministry selects Ngoc Linh ginseng to be come a National medicinal plant and national product pharmaceuticals. The government has issued the decision No 936/QD- TT (18/07/2012) approving the overall planning for economic-society development of Highland to 2020, which develop Ngoc Linh ginseng to be come the special national plant.

Since 2000, the Vietnam Ministry of Health, the Ministry of Science and Technology have cooperated with the People's Committees of Quang Nam and Kon Tum provinces organized many seminars and provided funding to carry out many projects in order to comprehensively research, conservation and development of this precious species in Vietnam. Number of studies on *Panax vietnamensis* var. *vietnamensis* have been carried out and have been published in various international journals and national conferences. However, there are still many difficulties in the production of seedlings, such as: there is no seed preservation technique, no optimal propagation process and technique, which makes the germination rate still quite low (about only 50-60%), there is still no standard nursery, so the seedlings grow slowly, and the quality is low, making the number of seedlings not enough to meet the needs of developing and expanding ginseng growing area. This also creates a loophole for many seeds of other *Panax* species whichs similar to *Panax vietnamensis* var. *vietnamensis*, unknown origin thereby making the seedling garden become unsatisfactory.

Korea is a country with has many years of experience in the field of research, cultivation, development and use of ginseng

(*Panax ginseng*). Korean ginseng has a history of cultivation for more than three hundred years and today it has become one of the 12 great symbols of Korean culture (after Kimchi only). The technique of preserving and propagating by a specific substrate (also known as a seed chip) is a technique that creates optimal conditions for seed germination, preserves seeds and promotes seed germination through processing of physics and chemistry have been studied and used in Korea since the 2000s [6, 7, 9]. The materials used to make seedchip are friendly environment materials such as peat, coir, moss, etc., and containina full range of nutrients promote germination as well as containing full nutrients, moisture that enough to help seedlings develop in the first stage. The seedchip structure has 3 layers. The top layer protects the seed from being approached by insects or animals. Seeds are placed in the second layer, the bottom layer contains enough nutrients to ensure the growth of seedlings (Fig 1).

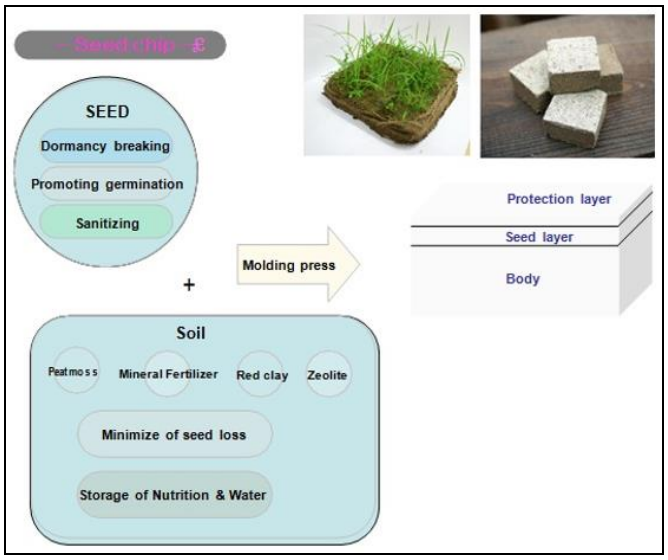

Fig 1: Seed chip structure (was made by research from Kyungpook University-Korea) [7]

Growing seedlings using Seed chip was developed in Korea for a long time, undergone many times of improvement and optimization, until now the size of a seed chip has been reduced to the optimal level (3x3cm). With this propagation technology, the germination rate of seeds is increased to the maximum. This technology has been granted patent number 10-920999 on October 1, 2009 in Korea [7]. Currently, propagation using specific substrates (seed chip) has been widely used in Korea, especially applied for the rare species whichs has low propagation rate from seeds in the natural environment.

In this study, we experimented of making seedchip according to the Korean procedure and experimented on creating seedlings for *Panax vietnamensis* var. *vietnamensis*.

Materials and methods

Materials

30 seeds of *Panax vietnamensis* var. *vietnamensis* were collected at Tac-ngo ginseng garden, Nam Tra My, Quang Nam (1875m, 15º09'N-107º54'E). Materials for making seedchip include coir mulch, moss and 10kg of humus soil taken in Tra Cang commune, Ngoc Linh. Chemicals include: Javen bleach (30%), potassium permanganate (KMnO4) 1%, growth regulators (NAA and IBA-K 98%), Bayfidan 25EC antifungal solution, water-retaining hydrogen gel beads (fine powder), liquid fertilizer (Bioplant flora).

Methods

Checking the collected seeds are exactly *Panax vietnamensis* by senquence the ITS gene.

Choosed randomly 5 seeds from 30 collected seed.

Extracted total DNA from seed coat by Dnaesy plant mini kit (qiagen).

ITS region with 700bp in length was amplified by primer pair ITS1 (5'-CCTTATCAYTTAGAGGAAGGAG-3')/ITS2 (5'-CGCCTTAKTGATATGCTTAAA-3') [2], Components of PCR reaction in 25 µl include: 12.5 µl PCR Master Mix, 2X (Thermoscientific, USA); 1 µl forward primer (10 pmol); 1 µl reverse primer (10 pmol); 1 µl DNA template (50 ng/ µl); 9.5 µl deionized H_2O. PCR reaction was carried out according to thermal cycle: 95oC for 6 minutes; 35 cycles (95oC for 30 seconds; 52oC for 30 seconds; 72oC for 1 minute 30 seconds), 72oC for 8 minutes; Store samples at 4°C. Checked PCR products by electrophoresis on 1% agarose gel.

Seed treatment

Choose the seeds: Fresh seeds, choose firm seeds, sink when soaked in water.

Seed treatment: Wash the selected seeds with clean water, then disinfect them by soaking in Javen water (NaOCl 30%) or potassium permanganate (KMnO4 1%) for about 50 minutes [9].

Processing of materials for the making seed chip

Sawdust, coir, dried moss are finely ground, then sterilization and mixed with water-retaining hydrogen gel beads, growth stimulant solution including NAA, IBA-K 98% until a rate of ~70% sawdust, coir., moss, ~10% is soil taken from Ngoc Linh, ~20% is a humectant (hydrogen gel beads retain water (fine powder form), (the mixture reaches ~15% moisture).

Results and Discussion

Result for checking collected seeds by ITS sequence

The results of PCR products by electrophoresis on 1% agarose gel showed that all 5 seed samples gave a clear band with size ~700bp as expected. They are proving that the ITS gene amplification process for all 5 samples may was successful. PCR products are clear, unbroken, eligible to perform direct sequencing (Fig 2).

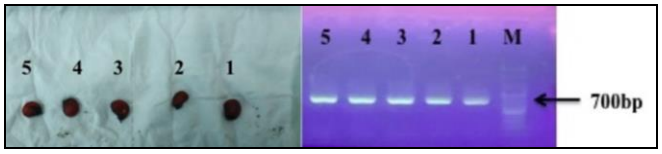

Fig 2: Electrophoresis of PCR products of 5 samples on 1% Agarose gel (M: DNA ladder 100bp (Fermentas, Lane 1-5: The samples from1 -5)

PCR products of 5 seed samples after purified by Exo-Alp PCR Cleanup Mix, then were used as template for direct sequencing with primer ITS1 (using Bigdye terminator cycler and reading results on ABI 3100 Avant Genetic Analyzer system (Applied Biosystems, USA). The sequencing result showed sharp and nice peaks (Fig 3).

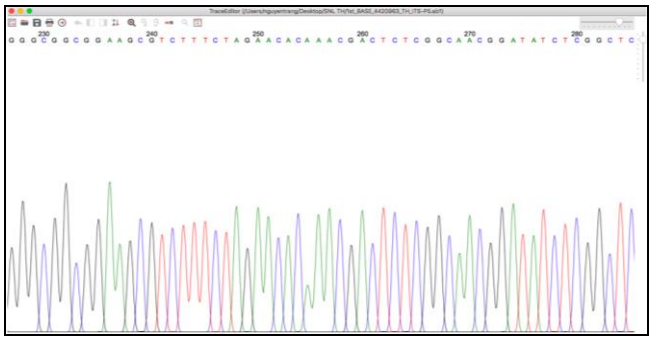

Fig 3: A part of image sequence of the sample number one

The gotten result checked on Blast application (www.blast.ncbi.nlm.nih.gov) showed that all of samples have the closest genetic relationship to *Panax vietnamensis* species (code MW741819) with genetic similarity is 100% (Fig 4).

Panax vietnamensis 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal RNA gene, internal tran...	Panax vietnamensis	1472	1472	100%	0.0	100.00%	7280	KT380922.1
Panax vietnamensis var. vietnamensis strain K5 small subunit ribosomal RNA gene, partial sequence; internal transc...	Panax vietnamen...	1472	1472	100%	0.0	100.00%	797	MW741819.
Panax vietnamensis strain K1 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5,...	Panax vietnamensis	1472	1472	100%	0.0	100.00%	797	MW741818.
Panax vietnamensis var. vietnamensis isolate 38 small subunit ribosomal RNA gene, partial sequence; internal trans...	Panax vietnamen...	1472	1472	100%	0.0	100.00%	6400	MW374467.
Panax vietnamensis var. langbianensis isolate DL-201918 small subunit ribosomal RNA gene, partial sequence; inter...	Panax vietnamen...	1472	1472	100%	0.0	100.00%	6400	MW374329.
Panax vietnamensis var. vietnamensis isolate 39 small subunit ribosomal RNA gene, partial sequence; internal trans...	Panax vietnamen...	1472	1472	100%	0.0	100.00%	6400	MW374330.
Panax vietnamensis var. langbianensis isolate DL-201935 small subunit ribosomal RNA gene, partial sequence; inter...	Panax vietnamen...	1472	1472	100%	0.0	100.00%	6400	MW374462.
Panax zingiberensis isolate JYH-2016439 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1,...	Panax zingiberensis	1456	1456	100%	0.0	99.62%	5874	MK408787.1
Panax zingiberensis isolate JYH-2016440 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1,...	Panax zingiberensis	1447	1447	100%	0.0	99.37%	5874	MK408788.1
Panax zingiberensis isolate JYH-2016464 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1,...	Panax zingiberensis	1445	1445	100%	0.0	99.37%	5874	MK408803.1

Fig 4: Results of comparison of 1st sample sequence on the Genbank

From above results, the collected samples are exactly *Panax vietnamensis* var. *vietnamensis* and can continue to be used for the next experiment.

Making seed chip based on the growth and development characteristics of *Panax vietnamensis*

The seed chip structure has 3 layers.

+ The materials for 3rd layer including sawdust, coir, moss, moist soil, after being mixed with growth stimulant solution with moisture ~15% are continued to be mixed with moisturizing hydrogen (2:1 ratio). The mixture then put into a mold to mold into a square form with thickness of 2-3 cm.

+ The middle layer (2nd layer): raw materials of coir, sawdust, moss, water-holding hydrogen powder and red soil incubated with a liquid fertilizer solution of Bioplant Flora (at the rate of 1/500). Then, the mixture to be wrapped in dumplings.

+ The outer layer (protective layer): a layer of humus soil has been mixed with antifungal solution and bactericide (Bayfidan 25EC), this covered has a thin about 0.1-0.2 cm (Fig 5).

The seeds after collect will be stored in the middle layer until using.

Fig 5: The seed chip used in preserving and growing seeds

Seeds after harvesting, removing floaters seeds (when soaked in water), disinfecting treatment by Javen water (NaOCl 30%) or potassium permanganate (KMnO4 1%) and drying, then put into the seed chip in absolute dry conditions, keep the chip (containing seeds-called seed chip) in vacuum-sealed plastic bags. According to the research report of Kyungpook University Korea, with this preservation method, seeds can be preserved for up to 10 years [7]. However, in this study, we only used to preserve the seeds from August (after harvesting) and proceed to germinate the seeds in next November.

Results for germination of *Panax vietnamensis* seed on seedchip

Among the 30 collected seeds of *Panax vietnamensis*, we selected 24 sturdy seeds to put into the seedchip. These seedchip then were preserved in vacuum plastic cover from August to Novembe, 2021. When conducting seed germination, the plastic cover was removed, the seedchip were immersed in water about 5-10 mins, then and kept in the incubator at 5-8°C, 80% humidity, the air circulation reaches a speed of 3.5m/s. After 70-80 days, the seeds begin to germinate and develop into seedlings (Fig 6).

Fig 6: Image of seeds germinating and growing into seedlings in an Incubator

Our experiment results show that 24/24 *Panax vietnamensis* seeds germinated (reaching 100%).

After 90-100 days grow under laboratory conditions, the seedlings reached a height about 15-21 cm, then they were planted in to the natural environment. 24/24 seedlings is planted under the canopy of a natural forest at the Tac-ngo ginseng garden, Nam Tra My district, Quang Nam province (Fig 7).

After 6 months of planting in a natural environment, 23/24 ginseng plants were still well alive (reach 95.83%) (There is one ginseng plant death but no disease was detected. maybe during the time for transportation from Hanoi to Quang Nam province, the plant has to become weak and when released it into the natural environment, the plant isn't strong enough to survive.

(Photo taken after 3 months of experimental planting in the natural environment (B))
(**A**. Seedlings in the laboratory, **B**. Seedlings in the natural environment)

Fig 7: Image of *Panax vietnamensis* planted in a natural environment in Nam Tra My, Quang Nam province

Specific biological seedchip are made from environment friendly materials. When put it into the soil, the seedchip will then decompose on its own.

The results of monitoring the seedlings after 6 months in the natural environment showed that the plants has healthy vitality and uniform growth compared with the plants grown from seeds in the natural environment at the beginning. Comparing the germination rate, 100% of seeds germinated and grown on seedchip in the laboratory condition (compared to ~60-69% with ginseng seeds when grown in the natural environment). The average growth rate of seedlings was 97.9% (compared to the control rate of 70-75% when seedlings grown in the natural environment).

For Korean Ginseng (*Panax ginseng*), this technique has brought the survival rate of seedlings up to 99%. In addition, recent studies in Korea have also shown that the seeds planted on a seed chip not only giving a high germination rate, but also have stronger vitality, faster growth and adaptation with environmental conditions better. The experiment comparing 2 batches of ginseng grown at Joung-Ju ginseng Garden (performed by technicians of Kyungpook National University) in the period 2010-2015 showed that the batch of ginseng grown on a seedchip gives the seed germination rate up to 99%, the plant grows healthy and gives roots larger nearly 2 times (2X) than the batch of ginseng grown from natural seeds under the same environmental conditions [7, 9].

The outstanding advantage of seedchip technology is growing seeds and creating seedlings becomes extremely easy and can be done in any area, not necessarily in the natural habitat of the plant. Seedlings grow stronger, more easily adapt to the conditions of the natural environment. Up to now, in Korea, the propagation technique on the seedchip not only applied to ginseng cultivation, is also being applied to some orchid species and many other valuable medicinal plants and the germination rates reaching over 20% compared to growing from seeds by traditional methods [2].

Conclusion

We have succeeded in creating "specific biological seedchip" folowing the published in patent No. 10-0920999 dated October 1, 2009 in Korea. Preliminary results show that the seedchip helps ginseng seeds have a high germination rate, ensuring good growth and development for seedlings before bringing the plant to its natural habitat. However, with a short time and a small number of experimental seeds, more time to monitor is necessary and continue experiment with large of seeds.

Acknowledgement

This research is funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number "106.06-2018.310".

References

1. Cheng T, Chao Xu, Li lei, Changchao Li, Yu Zhang, Shilian Zhou. Barcoding the kingdom plantae: New PCR primer for ITS region of plants with improved universality and specificity. Molecular Ecology Resources. 2016; 16:138-149.
2. CBOL Plant Working Group, A DNA barcoding for land plants. PNAS 106, No31, 2009, 12794-12797.
3. Chromas Pro2.1.6 (Technelysium Pty Ltd, Helensvale, Queensland, Australia).
4. Do Tat Loi. Vietnamese medicinal plants and herbs. Medicine Publishing House, 2004, 808-810. (In Vietnamese).
5. Le Thanh Son, Nguyen Tap. Basic ecological characteristics of Ngoc Linh Ginseng, Journal of Medicinal (in Vietnam). 2006; 11(4):145-147.
6. Lee B, Chongryol Park. Effects of Seed Coating, Slope Control and Soil Mulching on Seed Germination and Seedling Growth of Rehabilitation Plants. Journal of Korea Society of Environmental restoration technology. 2006; 25(4):456-466.
7. Hyoung Min Suh, Dong Geun Kim, Nam Sook Lee. Development of restoration technology using native plants seed chip for various disturbed slopes. Journal of Korea Society of Environmental restoration technology. 2010; 13(1):134-142.
8. Kumar S, Stecher G, Tamura K. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets, Molecular Biology and Evolution. 2016; 33(7):1870-1874.
9. Kyung Hoon Kim, Young Shin Kim, P Joo, Young Jin Hur. Seed germination study to determine the feasibility of local resources as materials for Ecological Restoration. Journal of Environmental Science. 2015; 18(1):153.
10. IUCN. The IUCN Red List of Threatened species, IUCN.SSC (WWW.Redlist.org), 2015.