RESEARCH ABILITY TO CREATE CALLUS AND REGENERATION PANAX BIPINNATIFIDUS (PANAX BIPINNATIFIDUS) IN VITRO CULTURE

Nguyen Nhu Toan[1*], Luu Ngoc Sinh[1], Nguyen Th Binh[1], Tran Đang Khanh[2]

[1] *Hanoi Metropolitan University*
[2] *Agricultural Genetics Institute*

Abstract: *Vu Diep ginseng is known to people as Tam That Wild, Tam That Leaf Split, Hoang Lien That, Tam That Lobe split bird feathers twice, Vu Diep Tam That, Ginseng Twice Split, Bamboo Blood Ginseng but no ginseng. Many international scientists note research on it. Studies show that Vu Diep ginseng contains a number of medicinal substances that are beneficial to health such as: saponin triterpen, Saponin A, B, C, D, reducing sugar, oleanolic acid and 16 amino acids such as lysine, cysteine, histidine, valine, phenylalanine, leucin, isoleucin, proline and inorganic substances such as Fe, Ca. In which, experts said that Vu Diep ginseng contains many compounds similar to ginseng. In particular, the leaves and roots, and flowers of Vu Diep ginseng contain saponoside compounds of the dammaran group. Vietnam is researching as well as producing, trying to awaken the medical and economic value of Ginseng Vu Diep. Our studies have initially determined the environment, influencing factors and the ability to create callus as well as the regeneration process of Invitro-environmental plants.*

Keywords: *Panax, Invitro, Callus, Embryo, Invitro, MS.*

Received 17 May 2022

Revised and accepted for publication 26 July 2022

(*) Email: nntoan@daihocthudo.edu.vn

1. INTRODUCTION

1.1. Materials and Methods

Ginseng Vu Diep is known to people as Tam That Wilderness… but not many international scientists pay attention to research on it. Studies show that Vu Diep ginseng contains a number of medicinal substances that are beneficial to health such as: saponin triterpen, Saponin A, B, C, D, reducing sugar, oleanolic acid and 16 amino acids such as lysine, cysteine, histidine, valine, phenylalanine, leucin, isoleucin, proline and inorganic substances such as Fe, Ca. In which, experts said that Vu Diep ginseng contains many compounds similar to ginseng. In particular, the leaves and roots, and flowers of Vu Diep ginseng contain saponoside compounds

of the dammaran group. Vietnam is researching as well as producing, trying to awaken the medical and economic value of Ginseng Vu Diep. In the research directions, the direction of tissue culture has really brought agriculture to an advanced stage, so if you want to research and develop Vu Diep ginseng in a modern direction, bringing high economic efficiency, you cannot ignore it. through this technique. In fact, Vu Diep ginseng has been successfully propagated from seeds and tubers... But going one step further to produce ginsenoside Vu Diep ginseng by tissue culture, almost no research works have been published. . With the desire to learn about this plant of economic value along with the cell culture technologies that have been and are being implemented for the purpose of propagation and production of compounds of economic value, we develop Research on the topic: "Study on the ability to create callus and regenerate seedlings of Vu Diep ginseng (Panax bipinnatifidus) in invitro culture medium".

The required purpose of the topic: Determining the ability to create callus, regenerate shoots and root in the process of creating seedlings of Panax ginseng (Panax bipinnatifidus) by tissue culture method; Creating quality seedlings, serving the needs of mass production of medicinal ginseng in a number of mountainous districts (Ba Vi, Soc Son) of Hanoi city and northern mountainous provinces

1.2. Material

Parts of Vu Diep Ginseng.

- Biological characteristics of Vu Diep ginseng plant

+ Scientific name: Panax bipinnatifidus

+ Family: Araliaceae family

+ Other names: Tam That leaves sawed, Hoang Lien ventricular, Tam That lobe split bird feathers twice, Vu Diep Tam That, Ginseng twice split, Bamboo details ginseng

It is a perennial herbaceous plant with a height of 10-20 cm, sometimes growing to a height of 50 cm. Compound leaves with stalks 6-8 cm long, hairless. Flowers grow in clusters at axillary stalks, white. The berries are a type of berry that usually grows in clusters and has a spherical shape. Inside the fruit contains 1-2 seeds and when ripe is red. The tubers are long, the inner intestine is yellow, white or purple. Wild tamarind is usually found in moist forests with altitudes from 1900 to 2400 m. The tree is commonly distributed in North Vietnam (many in Lao Cai) and Southern China. Parts Used: Root tubers. Harvesting and processing: The roots of perennial plants after being harvested will be washed and then dried or dried. Wild sage contains many saponins. In addition to these components, the plant also contains many medicinal substances similar to those in Ngoc Linh ginseng.

Environment:

Using MS background environment. Also added: 1.0 mg/l 2,4-D and 0.2 mg/l TDZ.

The culture medium was adjusted to pH = 5.8. The medium was sterilized by autoclave at 1210C, 1atm pressure.

Equipment and tools:

Room for preparation of medium, sterilization of culture medium, preservation of mother solution. Includes environmental autoclave, refrigerator, electric stove, analytical balance, measuring tube, pipette, pH meter.

- Aseptic inoculation room includes plant cabinets, UV lamps, autoclaved instruments.
- Cold room for culture includes iron shelves, lights, thermometers, air conditioners.
- Tools include alcohol lamp, plate, sample cutter, scissors, cotton ball, 250 ml, 500 ml sterile test tube bottles, sterile paper, elastic band.

Chemistry:

Alcohol 96^0, 70^0; Sterile distilled water; Javel solution; Dilute soap solution.

- Subsequent studies use the results of previous studies such as callus, shoots, etc.

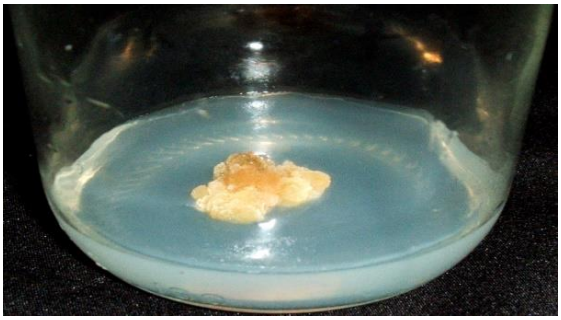

Figure 2.1. Sam Vu Diep callus

Figure 2.2. Vu Diep Ginseng Buds

Figure 2.3. Vu Diep Ginseng Root

Figure 2.4. Sam Vu Diep clonal embryo

2. RESEARCH METHODS

- The experiments are deployed and conducted according to the general procedure including:

+ Scar tissue culture

+ Regeneration of shoots from callus

+ Root culture

+ Cultivation of biomass

- *Plant regeneration through somatic embryogenesis*

Statistical analysis

+ Figures are calculated using Excel software.

+ Applying SAS software (2008) to analyze and compare experimental results.

+ The means were separated on the basis of the least significant differences (LSD) at the 0.05 probability level.

3. RESULTS AND DISCUSSION

3.1. Investigate the conditions affecting the culture of Vu Diep ginseng

3.1.1. Effects of some disinfectants on the culture:

For Sam Vu Diep, the experimental part is the head (germ, stem, and root).

Select straight or lateral shoots as culture material with a length of 2-3 cm, remove all leaves, treat with 70% alcohol for 1 min in a sterile incubator, then rinse 3 times with distilled water. then treat the sample with calcium hypochlorite or HgCl2 solution and continue rinsing with sterile distilled water several times to remove all disinfectant. After sterilization, cut the green head, length 0.4 cm, and place it in the medium.

Table 3.1. Effect of different types of sterilization concentration

Sample	**Sample number**	**$HgCl_2$**								
Sam Vu Diep (Sprout, stem, root)	10	0.2%				0.3%				0.4%
	Time (minute)	5	10	15	20	5	10	15	20	5
		100%	100%	100%	20%	40%	36%	Chết	Chết	Chết
Sample	Sample number	**$Ca(OCl)_2$**								
Sam Vu Diep (Sprout, stem, root)	10	5%			10%			15%		
	Time (minute)	10	15	20	5	10	15	5	10	
		100%	100%	100%	42%	Chết	Chết	Chết	Chết	

Vu Diep ginseng treated with HgCl2 at a concentration of 0.2% for 20 minutes, the infection rate was low (20%) and the infection rate was not high (80%). As for the treatment with Ca(OCl)2 at 10% concentration, for 5 minutes, the infection rate was low (42%) and the no infection rate was high (58%). (Table 3.1).

3.1.2. The influence of hormone combinations on morphogenesis

Using samples of sprouts, stems and roots of Vu Diep ginseng. Co-culture on different combinations of media to evaluate morphogenesis.

Table 3.2. Effects of hormone combinations on morphogenesis of Ginseng Vu Diep

Subject	**Implant parts**	**Hoccmon**						**The result of morphogenesis**
		IBA	**IAA**	**NAA**	**K**	**2ip**	**G**	
Ginseng Vu Diep	**Sprouts, Stems, Roots**	0.2			0.2			Died
		0.5			0.5			Died
		0.5			1			Callus
			0.2		0.5			Callus
			0.5		0.25			Roots
		0.5				1	1	Somatic embryo
		0.5				3	0.5	Bud embryo
				6		0.4		Roots
				1			3	Buds

The data in Table 3.2 shows that:

- Combinations of IBA 0.2 + 0.2 K and IBA 0.5 + 0.5 K cause death, combination IBA 0.5 +1.0K and combination IAA 0.2 + 0.5K create Callus. The remaining combinations such as IBA 0.5 + 2ip 1 hormone combination, then Sam Vu Diep generates somatic embryos; The combination of hormones NAA 1 + G 3 produces shoot embryos and the combination of hormones NAA 6 + 2ip 0.4 produces roots.

3.1.3. Effect of lighting conditions on the ability to create callus from leaves and petioles

The best medium for initial callus formation from leaf and petiole samples was used to investigate lighting conditions. Samples were placed in two conditions of complete darkness and light (16 h/day).

Depending on the type of explant, light may or may not be needed during callus formation. For leaf samples, in most cases, callus formation in the dark was usually better than in the light. However, in some cases, the explants produced better callus in bright conditions.

The results in Table 3.3 show that the rate of callus formation on leaf and petiole samples is almost equivalent between the two light and dark conditions, but the amount of callus in the dark condition is less and the callus quality is also poor. due to vitreous phenomenon, especially the medium with 3.0 mg/l 2,4-D.

Table 3.3. Effect of lighting conditions on the ability to create callus from leaves and petioles

2,4-D (mg/l)	Part	Percentage of callus formation (%)	
		Lighting (16 hours/day)	Totally dark
0.5	Leaves	20	30
1.0		90	80
2.0		90	90
3.0		80	80
0.5	Petiole	100	100
1.0		100	100
2.0		100	100
3.0		100	100

3.1.4. Effect of initial explant size on callus proliferation

Callus after proliferation was used for shoot regeneration and adventitious roots.

Callus was cut in three different sizes, respectively: KT1, KT2, KT3. Callus samples with defined size were inoculated into rapid multiplication medium.

Table 3.4. Effect of initial explant size on callus proliferation

Observation criteria		KT1 (0.5*0.5)	KT2 (0.8*0.8)	KT3 (1.0*1.0)
Original fresh weight (mg)		139 ± 8	268 ± 12	488 ± 19
Biomass after 4 weeks of culture	Dimensions (cm)	1.1*0.9	1.2*1.0	1.4*1.2
	Fresh weight (mg)	626 ± 38	812 ± 32	1516 ± 62
	Dry weight (mg)	51.9 ± 31	55.8 ± 2.3	112.6 ± 4.7
	Dry matter percentage (%)	8.18	7.08	6.59
Dry biomass growth rate		5.44	3.28	2.64

The explant size is an important factor in in vitro propagation. When investigating the effect of initial explant size on callus proliferation, we found that the smallest size (KT1) gave the best effect in terms of both biomass growth and dry weight, while not there was a big difference in proliferation ability between KT2 and KT3 (Table 3.4). This correlation can be derived from the correlation between the explant size - the ability to obtain nutrients from the medium and due to the influence of endogenous waste products of the callus during the culture process.

3.1.5. Effect of auxin on the ability to initiate callus from leaves and petioles

Studies available on subjects of the genus Panax have shown that the callus initiation phase often involves a combination of cytokinin and auxin.

After sterilization, leaf and petiole samples were inoculated into MS medium supplemented with 0.2 mg/l TDZ and auxins 2,4-D, IBA, NAA, with concentrations varying from 0.5; 1.0; 2.0 and 3.0 mg/l. The leaf specimen was placed face up on the medium and the petiole was also placed face up (Cut facing up). The results obtained after 8 weeks of culture are recorded in Table 3.5. Of the three types of auxins added to the medium, only 2,4-D was able to stimulate leaves and petioles to create callus. On medium supplemented with 1.0 mg/l 2,4-D, the explants had the highest rate of callus formation (reaching 90% for leaves and 100% for petioles), with the highest amount of scar formation. , firm structure and bright yellow color. At the concentration of 3.0 mg/l 2,4-D, the scar tissue started to show vitreous phenomenon. Therefore, at a concentration of 2,4-D of 3.0 mg/l or more, it is not suitable for callus generation.

Table 3.5. Effect of auxin on the ability to initiate callus from leaves and petioles

Auxin	**Auxin concentration (mg/l)**	**Scar tissue formation rate (%)**	
		Petiole	**Leaves**
2,4-D	0.5	100	20
	1.0	100	90
	2.0	100	90
	3.0	100	80
IBA	0.5	0	0
	1.0	0	0
	2.0	0	0
	3.0	0	0
NAA	0.5	0	0
	1.0	0	0
	2.0	0	0
	3.0	0	0

3.1.6. Effect of auxin on the ability to proliferate the callus of Sam Vu Diep

Callus samples generated from the initiation stage were inoculated into MS medium supplemented with 0.2 mg/l TDZ and auxins 2,4-D, IBA and NAA with concentrations varying from 0.5; 1.0; 2.0; 3.0 and 5.0 mg/l under irradiation conditions 16 h/day. The results obtained in Table 3.6 showed that: After the proliferation process, callus cultured on medium with 0.5 mg/l IBA had the highest dry matter ratio of 9.62% but the highest dry mass growth rate was 4.56 times. was obtained in callus on medium with 2,4-D at a concentration of 1.0 mg/l. It is possible that the combination of auxin and cytokinin increased the ability of the callus to obtain sugars and other nutrients from the callus environment and led to the proliferation of the callus, especially the dry matter ratio. IBA may be an auxin that stimulates nutrient uptake from the environment better when combined with TDZ than NAA and 2,4-D. As a result, the dry matter ratio of callus cultured on medium containing IBA was the highest among the three auxins used. Although IBA gave the highest percentage of callus with the highest percentage of dry matter,

2,4-D had the highest dry matter growth rate (4.56 times) and a relatively high dry matter rate (8.18%). On the other hand, callus on 2,4-D medium has the best morphology, which is a form of callus with high regenerative capacity.

Table 3.6. Effect of auxin on the ability to proliferate the callus of Sam Vu Diep

Auxin	Concentration (mg/l)	Original fresh weight (mg)	Biomass after 4 weeks of culture			Dry biomass growth rate
			Fresh weight (mg)	Dry weight (mg/l)	Dry matter percentage (%)	
2,4-D	0.5	203 ± 16	584 ± 34	43.3 ± 2.5	7.42	3.18
	1.0	212 ± 14	809± 37	66.2 ± 3.0	8.18	4.56
	2.0	204 ± 17	711 ± 32	52.4 ± 2.4	7.37	3.73
	3.0	205 ± 9	508 ± 24	36.6 ± 2.2	7.21	2.65
	5.0	201 ± 13	493 ± 38	34.6 ± 1.7	7.01	2.50
IBA	0.5	197 ± 18	474 ± 23	45.6 ± 2.2	9.62	3.45
	1.0	203 ± 19	532 ± 29	48.6 ± 2.7	9.14	3.56
	2.0	207 ± 13	631 ± 32	49.5 ± 2.5	7.84	3.63
	3.0	203 ± 15	552± 26	41.1 ± 1.9	7.45	3.10
	5.0	209 ± 12	531 ± 23	35.3 ± 1.5	6.66	2.53
NAA	0.5	218 ± 8	485 ± 13	41.2 ± 1.1	8.49	2.81
	1.0	212 ± 14	548 ± 21	45.0 ± 1.8	8.22	3.33
	2.0	206 ± 15	588 ± 18	46.6 ± 1.4	7.92	3.37
	3.0	199 ± 7	602 ± 32	45.7 ± 2.4	7.60	3.38
	5.0	205 ± 14	720 ± 48	51.6 ± 3.4	7.20	3.77

3.2. Some factors affect the ability to regenerate shoots from callus

3.2.1. Effect of BA and NAA on shoot regeneration from callus

Callus obtained in the callus rapid multiplication experiment were separated and transferred into ½ MS medium supplemented with BA and NAA with concentrations in Table 3.6. The ratio between auxin and cytokinin is essential for shoot regeneration, cytokinin usually promotes shoot formation and this process is often stimulated by the addition of auxin at low concentrations. In the trial, when using BA in combination with NAA, the results showed that different combinations of NAA and BA, the combination of 1.0 mg/l BA and 1.0 mg/l NAA gave the highest number of shoots at 6.3 buds/ sample and the mean weight was 0.185 g (Table 3.7).

Table 3.7. The ability to regenerate shoots from callus on MS . medium with additional BA and NAA

BA (mg/l)	NAA (mg/l)	Number of shoots/sample	Bud weight (g)
0.5	0.5	5.0	0.106
	1.0	6.1	0.141
	1.5	4.6	0.193
	2.0	3.3	0.197
	2.5	3.0	0.094
1.0	0.5	5.5	0.163
	1.0	6.3	0.185
	1.5	5.9	0.158
	2.0	3.9	0.148
	2.5	3.7	0.157
2.0	0.5	4.2	0.152
	1.0	5.5	0.141
	1.5	2.9	0.144
	2.0	2.8	0.112
	2.5	2.7	0.108
4.0	0.5	3.3	0.154
	1.0	3.0	0.122
	1.5	2.6	0.122
	2.0	0.8	0.108
	2.5	0	0

3.2.2. Effect of BA on shoot growth of Vu Diep ginseng invitro

The best shoots after collection were separated and transferred to ½ MS medium supplemented with 1.0 g/l activated carbon, 30 g/l sucrose, 0.5 mg/l NAA and BA (0.5; 1.0; 2.0; 4.0 mg/l).

Table 3.8. Effect of BA on growth Vu Diep ginseng buds invitro

BA (mg/l)	Trọng lượng tươi (g)	Chiều cao chồi (cm)	Số lượng lá/ chồi
0.5	0.61	5.66	3.0
1.0	0.87	6.16	3.3
2.0	0.72	4.11	4.0
4.0	0.71	4.33	3.9

Of the BA concentrations used, a concentration of 1.0 mg/l BA combined with 0.5 mg/l NAA resulted in the best shoot growth with shoot fresh weight of 0.87 g and shoot height of

6.16 cm (Table). 3.8). Therefore, the culture medium supplemented with 1.0 mg/l BA and 0.5 mg/l is best for shoot growth.

3.2.3. Effect of sugar concentration on shoot growth

The best shoots in the experiment were separated and transferred to ½ MS medium supplemented with 0.5 mg/l NAA, 1.0 mg/l BA, pH = 5.7 and sugar with sugar concentrations of 10; 20; 30; 40; 50; 60 g/l.

Table 3.9. Effect of sugar concentration on shoot growth

Sucrose (g/l)	Bud weight (g)	Bud height (cm)	Number of leaves/buds
10	0.49	4.4	2.2
20	0.55	5.4	2.5
30	0.68	5.7	2.6
40	1.06	5.8	3.2
50	1.46	6.1	3.5
60	1.28	6.1	3.2

The test results show that sucrose is the predominant soluble carbohydrate and the commonly used concentration is in the range of 30 - 120 g/l sucrose. Studying the effect of sucrose on shoot growth of Vu Diep ginseng after 90 days of culture showed that the addition of sucrose to the culture medium had a positive effect on shoot growth. The increase in sucrose concentration in the medium not only stimulates the growth of Vu Diep ginseng shoots but also has a strong effect on their weight change. A concentration of 50 g/l sucrose gave the best results in terms of weight, height and number of leaves (Table 3.9).

3.2.4. Effect of activated carbon on shoot growth in vitro

The best shoots in the experiment were separated and transferred to ½ MS medium supplemented with 0.5 mg/l NAA, 1.0 mg/l BA, pH = 5.7 with activated carbon concentrations of 1.0, respectively; 2.0; 3.0 and 4.0 g/l.

Activated carbon is not a plant growth regulator, but it has the ability to change the composition of the medium. Activated charcoal regulates the pH of the environment, absorbing substances that interfere with tissue growth. The obtained results showed that when the concentration of activated carbon increased, there was a clear change in the weight as well as the height of the shoots, but the number of leaves did not change significantly.

The highest shoot weight on the medium containing 2.0 g/l activated carbon was about 1.01 g/bud, an increase of 1.9 times compared to the control (Table 3.10). So the concentration of 2.0 g/l activated carbon is the most suitable for the proliferation of Vu Diep Ginseng buds.

Table 3.10. Effect of activated carbon on shoot growth in vitro

Activated carbon (g/l)	Bud weight (g)	Bud height (cm)	Number of leaves/buds
0	0.53	3.3	3.6
1.0	0.61	4.6	3.7
2.0	1.01	5.3	3.3
3.0	0.97	6.8	2.7
4.0	0.94	8.5	3.1

3.3. Factors affecting the possibility of uncertain rooting from callus

3.3.1. Effect of IAA, IBA, NAA on the ability of uncertain rooting from callus

Table 3.11. Effect of IAA, IBA, NAA on ability indeterminate rooting from callus

Auxin	Concentration (mg/l)	Rooting rate (%)	Amount roots / samples	Root mass/sample (%)	Maximum length of roots (mm)
NAA	1.0	30	3.0 ± 0.3	5.98	18
	3.0	100	8.7 ± 0.1	21.88	13
	5.0	70	2.6 ± 0.1	6.23	9
	7.0	50	2.1 ± 0.1	12.21	8
IAA	1.0	0			
	3.0	0			
	5.0	10	0.2 ± 0.2		
	7.0	0			
IBA	1.0	70	1.6 ± 0.1	7.83	16
	3.0	80	4.0 ± 0.3	5.21	21
	5.0	100	4.8 ± 0.3	15.81	18
	7.0	60	3.5 ± 0.1	8.06	1.7

Callus was inoculated into rooting medium containing auxins (NAA, IBA, IAA) at concentrations of 1.0, respectively; 3.0; 5.0; 7.0 mg/l. During the investigation of the effects of the above three types of auxins, we found that IAA is not suitable for the rooting of Vu Diep ginseng from callus, because this auxin hardly stimulates the callus to take root indeterminately. NAA and IBA are the opposite. Concentration of 3.0 mg/l NAA gave the best results with the rooting rate up to 100%, the largest number of roots/sample (8.7 roots/sample), the largest root-mass/sample ratio (21.88 %). The maximum length of the roots is 13 mm (Table 3.11). IBA concentration at 5.0 mg/l gave a 100% rooting rate, an average number of roots/sample of 4.8 samples, a mass ratio of 15.81% and a maximum root length of 18 mm. This result can be explained by the fact that the synthetic auxin is more active than the natural form. So two good rooting stimulators are Ms ½ medium supplemented with 3.0 mg/l NAA and MS ½ medium supplemented with 5 mg/l IBA.

3.3.2. Effect of IBA and NAA on the possibility of uncertain rooting:

Undetermined roots after being created in the experiments were separated and subcultured to rooting medium supplemented with auxins NAA, IBA at concentrations of 1.0, respectively; 3.0; 5.0 mg/l.

From the results in Table 3.12 and Table 3.13, the origin of the root sample has a great influence on the rooting efficiency. The root samples in Table 3.12 had better rooting ability, all 6 treatments took root, the highest rooting rate was 60%, the highest number of secondary roots was 9 roots/sample. The root samples in Table 3.13 had the highest rooting rate of 40%, the highest number of secondary roots was 3 roots/sample, 3 out of 6 rooting treatments but these samples were inoculated on medium containing NAA.

Table 3.12. Effect of IBA and NAA on rooting ability of samples derived from medium supplemented with NAA

NAA (mg/l)	IBA (mg/l)	Rooting rate (%)	Number of secondary roots	Average fresh weight (mg/l)
1	-	20	1	140 ± 10
3	-	30	4	290 ± 10
5	-	60	9	390 ± 20
-	1	10	1	450 ± 50
-	3	20	2	330 ± 20
-	5	30	1	280 ± 30

When considering the effect of auxin type, we find that NAA is more suitable for the uncertain rooting process of Vu Diep ginseng. At the concentration of 5.0 mg/l NAA stimulated the best root multiplication (60%), had the highest number of secondary roots (9 roots/sample) and high weight gain (average fresh weight was 390 ± 20 mg/l). , increased 3.5 times compared to the original). Furthermore, up to 5/6 treatments supplemented with NAA gave rooting results compared to IBA with only 4/6 treatments. So between IBA and NAA, NAA at a concentration of 3.0 mg/l is suitable for root induction from callus and NAA at a concentration of 5.0 mg/l is more suitable for uncertain root multiplication of Vu Diep ginseng.

Table 3.13. Effect of IBA and NAA on rooting ability of samples derived from IBA . supplemented medium

NAA (mg/l)	IBA (mg/l)	Rooting rate (%)	Number of secondary roots	Average fresh weight (mg/l)
1	-	40	3	350 ± 10
3	-	20	1	180 ± 30
5	-	0	0	
-	1	10	1	270 ± 10
-	3	0	0	
-	5	0	0	

4. CONCLUSION

Vu Diep Ginseng is recognized as one of the ginseng plants with high saponin content and the highest quantity, compared to other Panax species in the world. Therefore, the research and application of plant tissue culture technology has brought many practical meanings in conserving precious medicinal herbs.

- The process of investigating the effect of auxin type and concentration on the ability to create callus initially of leaves and petioles showed that concentrations of 3.0 mg/l 2.4-D or higher were not suitable for callus generation from leaves. Sam Vu Diep.

- During shoot growth, the number of shoots regenerated from callus was highest on ½ MS medium supplemented with 1.0 mg/l BA, 1.0 mg/l NAA, 50 g/l sucrose.

- For rooting from callus, callus samples were cultured on ½ MS medium supplemented with 3.0 mg/l NAA for the highest rooting rate, highest number of roots and fresh weight ratio. of the highest root/sample.

- ½ MS medium supplemented with 5.0 mg/l NAA stimulated the best root multiplication, giving the highest rooting rate and the most branching roots.

REFERENCES

1. Vu Thi Hien, Nguyen Phuc Huy, Bui Van The Vinh, Hoang Xuan Chien, Hoang Thanh Tung, Nguyen Ba Nam, Vu Quoc Luan, Duong Tan Nhut. 2015. *Somatic embryogenesis from leaf transverse thin cell layer derived-callus of Vietnamese ginseng (Panax vietnamensis Ha et Grushv.).* Tạp chí Công nghệ Sinh học số 1/2015, tr26.

2. Duong Tan Nhut, Nguyen Phuc Huy, Ngo Thanh Tai, Nguyen Ba Nam, Vu Quoc Luan, Vu Thi Hien, Hoang Thanh Tung, Bui The Vinh, Tran Cong Luan. 2015. *Light-emitting diodes and their potential in callus growth, plantlet development and saponin accumulation during somatic embryogenesis of Panax vietnamensis Ha et Grushv*. Biotechnology and Biotechnological Equipment, 29(2): 299-308.

3. Duong Tan Nhut, Hoang Thanh Tung, Vu Thi Hien, Nguyen Ba Nam, Nguyen Phuc Huy, Vu Quoc Luan. 2016. *Assessment of the possibility of flowering, fruiting and saponin accumulation of somatic embryo-derived Panax vietnamensis Ha et Grushv plants growing in kon tum and quang nam. Tạp chí Công nghệ Sinh học 14(1A): 263-268.*

4. Dương Tấn Nhựt (2014), Hoàn thiện quy trình nhân giống sâm Ngọc Linh hướng đến xây dựng thương hiệu Quốc gia, Viện hàn lâm khoa học và công nghệ Việt Nam, www.vast.ac.vn

5. Ngo Thanh Tai, Nguyen Ba Nam, Ho Thanh Tam, Ha Thi My Ngan, Duong Tan Nhut. two thousand and thirteen. Studying the effects of LED light on callus proliferation and complete plant formation from Ngoc Linh ginseng clones (Panax vietnamensis Ha et Grushv.). Proceedings of the Biotechnology Conference, Hanoi, 1038-1042.

NGHIÊN CỨU KHẢ NĂNG TẠO MÔ SẸO VÀ TÁI SINH CÂY SÂM VŨ ĐIỆP (PANAX BIPINNATIFIDUS) TRONG MÔI TRƯỜNG NUÔI CẤY INVITRO

Tóm tắt: *Sâm Vũ Diệp được con người biết đến với tên gọi Tam thất hoang, Tam thất lá xẻ, hoàng liên thất, tam thất thùy xẻ lông chim hai lần, vũ diệp tam thất, sâm hai lần chẻ, trúc tiết nhân sâm nhưng không có nhiều nhà khoa học quốc tế lưu ý nghiên cứu về nó. Các nghiên cứu cho thấy trong Sâm Vũ Diệp có chứa một số dược chất có lợi cho sức khỏe như: saponin triterpen, Saponin A, B, C, D, đường khử, acid oleanolic cùng 16 acid amin như lysine, cysteine, histidine, valin, phenylalanin, leucin, isoleucin, prolin cùng các chất vô cơ như Fe, Ca. Trong đó các chuyên gia nhận định Sâm Vũ Điệp có chứa nhiều hợp chất giống với nhân sâm. Đặc biệt, các bộ phận lá và rễ, hoa Sâm Vũ Điệp đều chứa các hợp chất saponosid nhóm dammaran. Việt Nam đang nghiên cứu cũng như sản xuất, cố gắng đánh thức giá trị y học và giá trị kinh tế của Sâm Vũ Diệp. Các nghiên cứu của chúng tôi đã bước đầu xác định được môi trường, các yếu tố ảnh hưởng và khả năng tạo mô sẹo cũng như quá trình tái sinh cây trong môi trường Invitro.*

Từ khóa: *Sâm, Mô tế bào, Nuôi cấy Invitro, Mô sẹo, Chồi, Môi trường nuôi cấy*