Micropropagation of Seabuckthorn *(Hippophae rhamnoides* L.)

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SUMMARY

Studies on the development of protocol was conducted on the Lahaul form of *H. rhamnoides* ssp. *turkestanica),* the most promising form of the Himalayas. With active bud explants of seabuckthorn, more than 95 per cent contamination free cultures were established through surface sterilization scheme of 0.1 per cent detergent (2 Hours), tetracycline (2 Hours), 70 per cent EtOH (4 Min) and 0.1 per cent $HgCl_2$ (6 Min), whereas with dormant buds 0.1 per cent detergent (5 Hours), Tetracycline (over night), 70 per cent EtOH (15 Min) and 0.1 per cent $HgCl_2$ (18 Min). There was an increase in overall explant survival from MS through ½ MS to WPM, and decrease in explant vitrification from through MS to WPM. Vitrification level decreased from MS to WPM. Comparison of media-hormone interaction revealed that explant survival was highest, on WPM medium (80.6 per cent), closely followed by ½ MS (80 per cent) and lowest on MS medium. Overall on MS medium, per cent multiple shoot, shoot/explant and callusing decreased along successive passages. On ½ MS maximum shoots/explant of a maximum of 1.4 shoots/explant was observed in combination BAP 0.2: IBA 0.01 in 60 per cent of the cultures, followed by maximum of 1.1 shoots/explant in BAP 0.2: IBA 0.01 in 40 per cent of the cultures during P 1. On WPM medium, in 60 per cent of the cultures, shoots/explant with a maximum of 14.6 shoot/explant was observed in BAP 1.0: IAA 0.5 ppm, which was followed by 66.7 per cent cultures having maximum of 5 shoot/explant in BAP 0.3: NAA 0.2 ppm combination during P 1. Overall there was increase in per cent multiple shooting, shoots/explant and callusing along successive passages on WPM medium. Comparison of multiple shoot development on different culture media revealed that there was increase in per cent multiple shoot development and shoot/explant across different culture media from MS through ½ MS to WPM along successive passages. Maximum shoot survival of 83.3 per cent was observed with IBA 1.0 ppm. Highest root induction of 66.7 per cent was observed with IBA 1.5 ppm.

Among different culture media tested with various growth hormone combinations, WPM medium with 3 per cent sucrose, was found to be suitable for the induction of multiple shoots, with hormone combination of BAP 1.0: IAA 0.5 ppm and WPM with 2 per cent sucrose and 1.5 ppm IBA was found to be suitable for the induction of rooting in seabuckthorn shoots. Further work is in progress to improve the multiple shoot frequency as well as improvement of rooting induction.

Keywords: Seabuckthorn, Explant, Phytohormones, Buds, MS, WPM and vitrification, Multiple shoots formation and Rooting.

INTRODUCTION

Seabuckthorn *(Hippophae rhamnoides* L.) is a hardy, deciduous shrub belongs to the family *Elaeagnaceae* (Rousi, 1971). It bears yellow or orange to red berries, which has been used for centuries in Europe and Asia due to the medicinal and nutritional properties (Singh, 2006). The natural habitat of seabuckthorn extends widely from cold regions of China, Himalayas, Mongolia, and central Asia to Russia, Great Britain, France, Denmark, Netherlands, Germany, Poland, Finland, Sweden and Norway (Singh, 2003). Seabuckthorn develops extensive root system in a short period of time, therefore it is planted for soil conservation and erosion, strengthen sandy slopes. It also has

been used in soil reclamation for its ability to fix nitrogen and conserve other essential nutrients (Li arid Schroeder, 1996; Lu, 1992). Seabuckthorn is a unique and valuable plant currently being cultivated in various parts of the world, including Canada. It can withstand temperatures from -43°C to +40°C and is considered to be drought resistant (Lu, 1992).

Micropropagation or tissue culture is a convenient, fast technique for producing high quality, clonally uniform and genetically identical, axenic plantlets of many desirable tree species. The technique facilitates year-round production capability and requires less space. It has immense potential in mass propagation in a genetic improvement programme of seabuckthorn, particularly, where the number of plus trees selected are limited for raising large plantations. The present paper discusses the research conducted by us on the micro-propagation of Indian seabuckthorn *(Hippophae rhantnoides* ssp. *turkestanica),* which grows in Indian Himalayas.

MATERIAL AND METHODS

Harvesting of Buds

Active buds of seabuckthorn were harvested from 2 years old seabuckthorn plants raised from cuttings under green house at CSK Himachal Pradesh Agricultural University's Hill Agricultural Research and Extension Center, Bajaura (Kullu), from March onward at an interval of two to three weeks. Dormant buds were collected during the end of October. The buds were harvested in 1500 ppm solution of ascorbic acid and citric acid each, which was decanted after one hour and then buds were stored at 4-6°C temperature in the refrigerator for 6 days.

Surface Sterilization

Active Buds

Active buds were surface sterilized using 0.1% detergent solution of teepol in 1500 ppm in the refrigerator. The buds were thoroughly washed with plenty of running tap water to remove traces of detergent. After detergent washing, the buds were treated with tetracycline solution (250 mg/100 ml) for two hours at low temperature. Then the buds were rinsed with 200 ml distilled water three times to remove the traces of tetracycline. After antibiotic treatment, buds were treated with 70 per cent EtOH for 4 minutes in the laminar flow hood and rinsed twice with 200 ml sterile distilled water. Finally the buds were treated with 0.1 per cent $HgCl_2$ solution for 6 minutes. After $HgCl_2$, the buds were rinsed thoroughly with sterile 200 ml distilled water for five times and kept in 1500 ppm solution of ascorbic acid and citric acid, till the preculturing.

Dormant Buds

Dormant buds were surface sterilized using 0.1 per cent detergent solution of teepol in 1500 ppm solution of ascorbic acid and citric acid each for 5 hours with gentle swirling of flask at low temperature in the refrigerator. The buds were thoroughly washed with plenty of running tap water to remove traces of detergent. After detergent washing, the buds were treated with tetracycline solution (250 mg/100 ml) over night at low temperature. Then the buds were rinsed with 200 ml distilled water three times to remove the traces of tetracycline. After antibiotic treatment, buds were treated with 70 per cent EtOH for 15 minutes in the laminar flow hood and rinsed twice with 200 ml sterile distilled water. Finally the buds were treated with 0.1 per cent $HgCl_2$ solution for 18 minutes. After $HgCl_2$ the buds were rinsed thoroughly with sterile 200 ml distilled water for five times and kept in 1500 ppm solution of ascorbic acid and citric acid till the preculturing.

Preculturing

Active and dormant buds were precultured on 1.2 per cent plain agar, pH 5.8, supplemented with 100 ppm Inositol and 3 per cent sucrose, for two weeks under 16/8 hours photoperiod at 18°C.

Culturing for Multiple Shoot Formation

After preculturing, the active and dormant buds were cultured on WPM, MS and ½ MS culture media, containing appropriate vitamins, various combinations of auxins and cytokinins with 1.2 per cent agar, pH 5.8, supplemented with 100 ppm Inositol and 3 per cent sucrose for induction of multiple shoots. Cultures were kept under 16/8 hours photoperiod at 18°C, and sub-cultured on to fresh medium every 4-6 weeks, with identical hormone and medium composition.

Culturing for Rooting

Multiple shoots were sub-cultured on to rooting medium with appropriate vitamins and various concentration of IBA, 0.8 per cent agar, pH 5.8, supplemented with 100 ppm Inositol and 2 per cent nucrose. Cultures were kept under 16/8 hours photoperiod at 18°C for 4 to 6 weeks.

RESULTS

Phenolics Accumulation

When the dormant buds as well as active buds were inoculated on to the MS full strength culture medium to begin with, a severe problem of phenolics accumulation and subsequent killing of all the explants was encountered. To find out the appropriate pH of the culture medium, culture medium with different pH was tried to see its effect on the accumulation of phenolics, which are summarized In the Table 1.1.

Table 1.1: Effect of pH on Phenolic Accumulation

pH	***5.5***	***5.75***	***6.0***	***6.25***	***6.50***	***6.75***	***7.0***	***7.25***
Apical buds	1.66	1.25	1.91	1.41	1.33	1.58	1.75	1.91
Dormant buds	1.83	1.33	1.58	1.66	1.81	1.75	1.91	2.36

Note: On a visual scale of 1 to 5 (Average mean of 12 replications).

For active and dormant buds, the best pH was found to be 5.75, as at this pH, the phenolics accumulation was minimum. Phenolics accumulation increased with increase in pH. Overall trend was increase in phenolics accumulation with increase in media pH.

To further over come the problem of phenolics accumulation by the explants, pretreatment of the explant with 1500 ppm ascorbic acid and citric acid solution (one hour) followed by low temperature treatment of explant (4-5°C, 5-6 days) and subsequent pre culture of explant on plain agar supplemented with sucrose and inositol (3 per cent Sucrose, 100 ppm inositol, pH 5.75-5.8) for two weeks was adopted.

It was found that chemical pretreatment alone without any cold treatment reduced the phenolics accumulation to minimum level when explants were inoculated on to the plain agar. Phenolics accumulation was maximum in MS medium, compared to WPM medium. Even after 144 hours of cold treatment, there was still accumulation of phenolics both in WPM and MS medium. There was no phenolics accumulation in plain agar after pretreatment and cold treatment of explants. This procedure helped in controlling phenolics accumulation as well as breaking the dormancy of more than 90 per cent of the dormant buds. These measures are summarized in Table 1.2.

Table 1.2: Remedial Measures to Overcome Phenolic Accumulation

Sl.No.	*Treatment*	*Concentration/Quantity*	*Duration*
1.	Ascorbic acid + Citric acid w/v in sterile DDW	1500 ppm each	One hour
2.	Low temperature	4-5 "C	5-6 days
3.	Pre culture of explant on plain Agar supplemented with sucrose and inositol	3 per cent Sucrose, 100 ppm inositol, pH 5.75-5.8	Two weeks

Explant Vitrification

To overcome the problem of vitrification of explants, the explants were cultured on culture media *i.e.* MS (Full strength), MS (Half Strength) and WPM (Full strength) to see the effect of culture media on vitrification and explant survival.

On MS Medium

On MS medium, across the different hormone combinations (BAP + NAA, BAP + IAA, BAP + IBA), vitrification levels ranged from 0 to 3 (on a visual scale of 5). There was no vitrification with combination BAP 1.0: IAA 0.5, whereas combinations BAP 0.2: NAA 0.05 and BAP 0.5: IAA 1.0 resulted in highest level of vitrification in up to 20 per cent of the cultures.

Overall, 45 per cent of the cultures had varying levels of vitrification and 55 per cent cultures were normal. The explant survival rate varied from 20-80 per cent. Combinations BAP 1.0: IAA 0.5, BAP 0.05: IAA 2.0 and BAP 0.1: IBA 0.5 had highest mortality with up to 80 per cent dead cultures. The overall explant survival was 50 per cent.

On ½ MS Medium

On ½ MS medium, across the different hormone combinations (BAP + NAA, BAP + IAA, BAP + IBA), vitrification levels ranged from 0 to 2 (on a visual scale of 5). Overall, 68.3 per cent of the cultures bad varying levels of vitrification and 31.7 per cent cultures were normal. Combinations BAP 0.2: NAA 0.3, BAP 0.3: NAA 0.1, BAP 0.2: NAA 0.05, BAP 0.5: IAA 1.0 and BAP 0.1: IBA 0.5 had highest level of vitrification ranging from 20-40 per cent of the cultures. The explant survival rate varied from 10-100 per cent. Combinations BAP 0.2: IBA 0.01 and BAP 0.3: NAA 0.1 had 100 per cent explant survival. The overall explant survival was 70 percent.

On WPM Medium

On WPM, across the different hormone combinations (BAP + NAA, BAP + IAA, BAP + IBA), vitrification levels ranged from 0 to 2 (on a visual scale of 5). Combinations BAP 0.3: NAA 0.1, BAP 0.2: NAA 0.3, BAP 0.5: IAA 1.0, BAP 0.05: IAA 2.0, BAP 0.5: IBA 0.1 and BAP 0.01: IBA 0.2 had highest level of vitrification ranging from 12.5 per cent to 62.5 per cent of the cultures. Overall, 42.4 per cent of I he cultures had varying levels of vitrification and 57.6 per cent cultures were normal. The explant Kin vival rate varied from 62.5-100 per cent. Combination BAP 0.5: IBA 0.1 had 100 per cent explant survival. The overall explant survival was 75 per cent.

General trend was an increase in overall explant survival from MS through ½ MS to WPM, decrease in explant vitrification from ½ MS through MS to WPM, vitrification level decreased from MS to WPM.

Comparison of media-hormone interaction revealed that explant survival was highest, on WPM medium (80.6 per cent), closely followed by ½ MS (80 per cent) and lowest on MS medium. In all the three group combinations of hormones (BAP: NAA, BAP: IAA, BAP: IBA) used, the overall trend was that on MS medium there was lowest explant survival.

Multiple Shoots Formation

On MS Medium

On MS medium, during P 0 passage, per cent multiple shoot ranged from 0 to 40 per cent across the different hormone combinations (BAP + NAA, BAP + IAA, BAP + IBA). Highest multiple shoots in 40 per cent of the cultures were observed in BAP 0.5: IAA 1.0 combination. Overall 11.7 per cent of the P 0 cultures had multiple shoots ranging from 0 to 0.4 shoots/explant. Across different hormone treatments average number of shoots/explant was 0.2. Callusing ranged from 0 to 20 per cent across different hormone combinations. In hormone combination BAP 0.2: IBA 0.01, callusing was observed in 20 per cent of the cultures. Overall 1.7 per cent of the P 0 cultures had callusing.

In P 1 passage cultures, percent multiple shoots ranged from 0-20 per cent across different hormone combinations. In hormone combinations: BAP 0.2: NAA 0.05 and BAP 2.0: IAA 0.05, 20 per cent of the cultures had multiple shoots formation. Overall, 3.3 percent P 1 cultures had multiple shoots formation ranging from 0-0.2 shoots/explant. Average shoots/explant was 0.03. In P 1 cultures, there was no callusing. Since, there was no multiplication of the explants after P 1 passage, therefore further sub culturing was not carried out beyond P 1 passage on MS medium. Overall trend with MS medium was an decrease in per cent multiple shoot, shoot/explant and callusing, along successive passages.

On ½ MS Medium

On ½ MS medium, during P 0 passage, percent multiple shoot ranged from 0 to 60 per cent across different hormone combinations (BAP + NAA, BAP + IAA, BAP + IBA). Maximum multiple shooting 26.7 per cent of the P 0 cultures had multiple shoots ranging from 0 to 1.4 shoots/explant. In 60 per cent of the cultures, shoots/explant of a maximum 1.4 shoots/explant was observed in combination BAP 0.2: IBA 0.01 (Plates 1.1 a, b and c). Average shoots/explant was 0.4. In P 0 cultures callusing ranged from 0 to 60 per cent across different hormone combinations. Maximum of 60 per cent callusing was observed in hormone combination BAP 0.1: IB A 0.5. Overall 15.0 per cent of the P 0 cultures had callusing.

In P 1 passage cultures, multiple shoots ranged from 0-60 per cent across different hormone combinations. Overall, 21.7 per cent of the P 1 cultures had multiple shoots ranging from 0-1.1 shoots/explant. In hormone combinations BAP 0.2: NAA 0.3, multiple shooting was observed in 60 per cent cultures. In combination BAP 0.2: IBA 0.01,40 per cent of the cultures had a maximum of 1.1 shoots/explant. Average shoots/explant was 0.4. There was no callusing.

In P II passage cultures, multiple shoots ranged from 0-40 per cent across different hormone combinations. Overall, 17.5 per cent of the P II cultures had multiple shoots ranging from 0-0.9 shoots/explant. Average shoot/explant was 0.2. In hormone combinations BAP 1.0: LAA 0.5, multiple shooting was observed in 40 per cent of the cultures. There was 0-20 per cent callusing. Overall 1.7 per cent of the cultures had callusing. In combination BAP 0.3: NAA 0.2, callusing was observed in 20 per cent of the cultures.

In P III passage cultures, multiple shoots ranged from 0-26.7 per cent across different hormone combinations. Overall, 13.6 per cent of the P III cultures had multiple shoots ranging from 0-0.5 shoots/explant. Average shoots/explant was 0.2. In hormone combination: BAP 1.0: IAA 0.5, multiple shoots formation was observed in 26.7 per cent of the cultures. There was 0-15.4 per cent callusing. Overall 1.3 per cent of the cultures had callusing. In combination BAP 0.3: NAA 0.2 callusing was observed in 15.4 per cent of the cultures. Overall trend with ½ MS medium was decrease in per cent shoots formation, shoots/explant and callusing along successive passages.

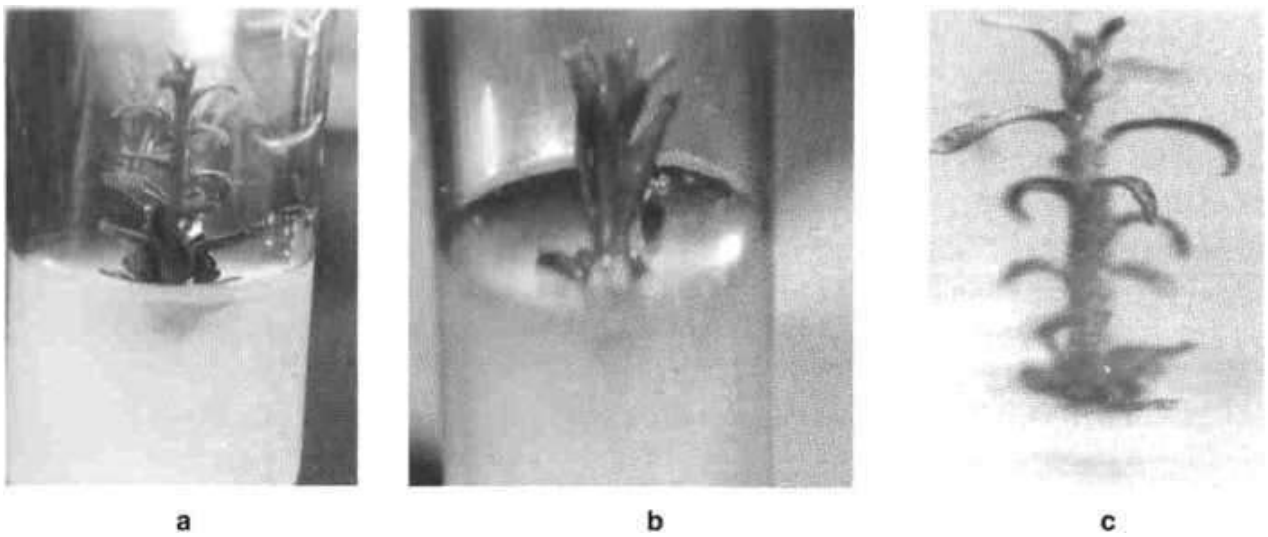
On WPM Medium

On WPM medium, during P 0 passage, per cent multiple shoots ranged from 0 to 60 per cent across the different hormone combinations (BAP + NAA, BAP + IAA, BAP + IBA). In combination: BAP 0.2: NAA 0.05 ppm, 60 per cent of the cultures developed multiple shoots. Overall 6 per cent of the P 0

cultures had multiple shoots. Shoots/explant ranged from 0 to 0.6. Average shoot/explant was 0.1. There was no callusing in P 0 cultures.

In P 1 passage, multiple shoots ranged from 0-66.7 per cent across different hormone combinations. Overall, 17.7 per cent of the P 1 cultures had multiple shoots. In hormone combinations BAP 0.3: NAA 0.2, multiple shooting was observed in 66.7 per cent cultures. Shoots/explant ranged from 0-14.6 shoots/explant. Average shoots/explant was 1.7. In 60 per cent of the cultures, shoots/explant with a maximum of 14.6 shoot/explant was observed in combination BAP 1.0: IAA 0.5 ppm, which was followed by 66.7 per cent cultures having maximum of 5 shoot/explant in combination BAP 0.3: NAA 0.2 ppm. Callusing ranged from 0 to 60 per cent across different hormone combinations. Overall in 13.3 per cent cultures, callusing was observed. In BAP 1.0: IAA 0.5 combination, 60 per cent cultures had callusing.

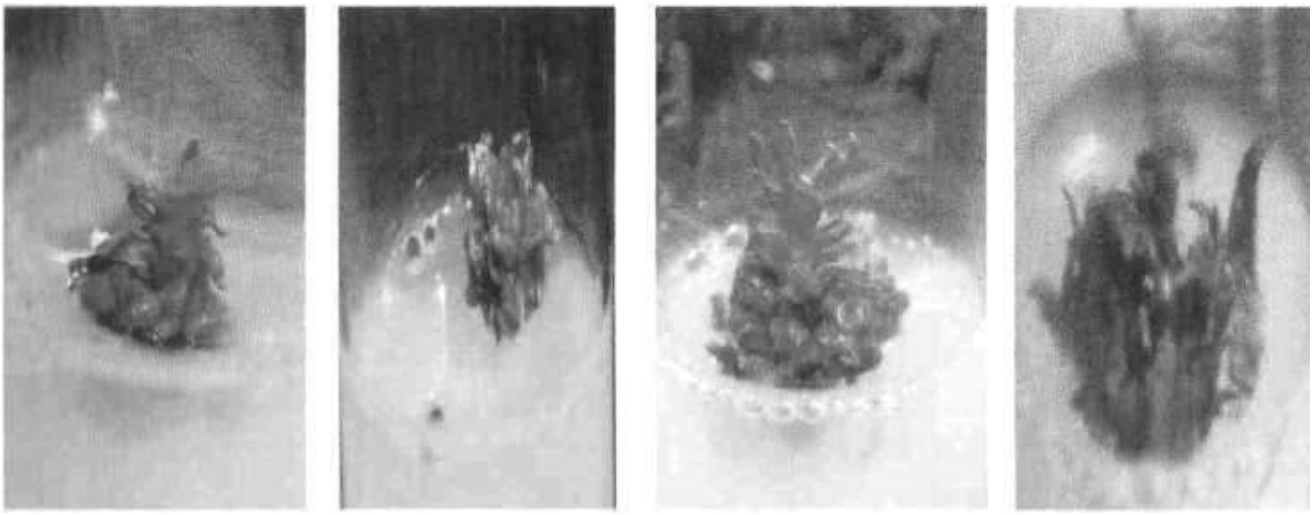
In P II passage cultures, multiple shoots ranged from 0-80 per cent across different hormone combinations. Overall, 15 per cent of the P II cultures had multiple shoots. In hormone combinations BAP 1.0: IAA 0.5 ppm, multiple shooting was observed in 80 per cent of the cultures with maximum of 6.5 shoots/explant, which was followed by 30 per cent cultures in combination BAP 0.3: NAA 0.2 ppm with maximum of 1.7 shoots/explant (Plates 1.2a, b, c and d). Overall, multiple shoots ranged from 0-6.5 shoots/explant. Average shoots/explant was 0.7. There was 0-60 per cent callusing. Average caIlusing was 9.2 per cent. In combinations BAP 1.0: IAA 0.5, callusing was observed in 60 per cent of the cultures.



Plates 1.1a and b: Development of Multiple Shoots;

Plate 1.1 c: Elongation of Subcultured Shoot on VfeMS

In P III passage cultures, only combination BAP 0.3: NAA 0.2, BAP 1.0: IAA 0.5, and BAP 0.5: IAA 1.0 were carry forward. Across these three combinations, multiple shoots ranged from 20-80 per cent. (Jverall, 42.2 per cent of the P III cultures had multiple shoots. In hormone combinations BAP 1.0: IAA 0.5, multiple shooting was observed in 80 per cent of the cultures with maximum of 8.7 shoots/explant. Overall, multiple shoots ranged from 0.3-8.7 shoots/explant. Average shoot/explant was 3.1 in these three combinations. Overall there was callusing in 17.8 per cent of the cultures. In combination BAP 0.3: NAA 0.2, callusing was observed in 26.7 per cent of the cultures.



a b c d

Plates 1.2 a and b: Development of Multiple Shoots with BAP 0.3 + NAA 0.2 ppm on WPM

Plates 1.2 c and d: Development of Multiple Shoots with BAP 1.0 + IAA 0.5 ppm on WPM

In general across all the tested hormone combinations with WPM medium along the successive passages, per cent multiple shoot development varied from 6 to 42.2 per cent. Multiple shoots/explant ranged from 0.1 to 3.1 shoots/explant. Callusing ranged from 0 to 17.8 per cent.

Overall trend was increase in per cent multiple shooting, shoots/explant and callusing along successive passages on WPM Medium. Overall trend with WPM was decrease in per cent multiple shoots along the passages with exception of BAP:IAA group. Overall trend with WPM was increase in shoots/explant along the passages.

Comparison of multiple shoot development on different culture media showed that during successive passages with BAP 0.3: NAA 0.2 hormone combination, a maximum of 66.7 per cent cultures developed multiple shoots in WPM medium (P II passage), followed by 40 per cent in ½ MS (P 0 passage), while there was no shoot development in MS medium.

In case of BAP 1.0: IAA 0.5 combination, a maximum of 80 per cent cultures developed multiple shoots in WPM medium (P II and P III passages), followed by 60 per cent cultures in WPM medium (P I passage), which was followed by 40 per cent cultures in ½ MS (P II passage), whereas there was no shoot development in MS medium.

On the other hand with BAP 0.5: IAA 1.0 hormone combination, 40 per cent cultures both in MS (P 0 passage) and WPM (P II passage) developed multiple shoots, followed by 20 per cent cultures in WPM (P III passage) and 10 per cent cultures in ½ MS (P II & P III). There was no multiple shoot development after P 0 passage in MS medium. With all the three hormone combinations, there was no development of multiple shoots in WPM medium (P 0 passage) and multiple shoot development started from P 1 passage with BAP 0.3: NAA 0.2 and BAP 1.0: IAA 0.5 combinations. With BAP 0.5: IAA 1.0, the multiple shoots started developing both in WPM and ½ MS from P II passage.

The overall trend was increasing in per cent multiple shoot development across different culture media from MS through ½ MS to WPM along successive passages.

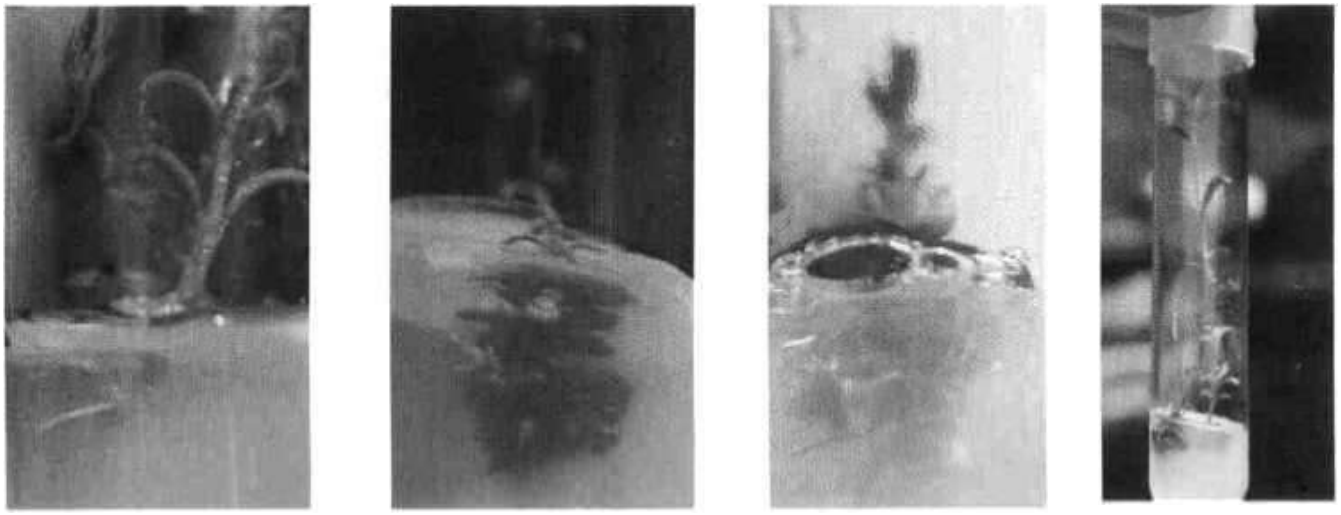


Plate 1.3: Root Induction on WPM Rooting Medium with IBA 1.5 ppm

Comparison of development of shoot/explant on different culture media showed that, during passages with BAP 0.3: NAA 0.2 hormone combination in WPM medium, a maximum of 5 shoots/explant developed (P 1 passage), followed by 1.7 shoot/explant (P Π), while there was no shoot development in MS medium.

In combination: BAP 1.0: IAA 0.5 in WPM medium, a maximum of 14.6 shoots/explant developed (P 1 passage), followed by 6.5 shoots/explant (P II passage), followed by 8.7 shoots/explant (P III) while there was no shoot development in MS medium (Table 1.3).

Table 1.3: Multiple Shoots/Explant During Successive Passage on Different Culture Media

Treatment	***MS***		***½ MS***				***WPM***			
	P 0	***P 1***	***P 0***	***P 1***	***P II***	***P III***	***P 0***	***P 1***	***P II***	***P III***
BAP 0.3: NAA 0.2	0	0	0.6	0.3	0.3	0.2	0	5	1.7	0.4
BAP 1.0: IAA 0.5	0	0	0.2	0	0.9	0.5	0	14.6	6.5	8.7
BAP 0.5: IAA 1.0	0.4	0	0	0	0.1	0.1	0	0	0.4	0.3
Average Shoots/ explant	0.13	0	0.27	0.1	0.43	0.27	0	6.5	2.9	3.13

On the other hand in BAP 0.5: IAA 1.0 combination with MS, 16MS and WPM, the shoot/explant development during different passage ranged from 0 to 0.4 shoot/explant. In general shoot/explant ranged from 0 to 6.5 shoots/explant.

Overall the trend was increase in shoot/explant across different culture media from MS through ½ MS to WPM along successive passages.

On ½ MS medium, explant shoot length ranged from 3.8 to 8.8 mm across different hormone combinations. Average shoot length ranged from 5.3 to 6.4 along the passages. In hormone combination BAP 0.2: IBA 0.01, a maximum shoot length of 8.8 mm was observed, while in combination BAP 0.01: IBA 0.2, minimum shoot length of 3.8 was observed. General trend was decrease in shoot length along the passages on ½ MS medium.

On WPM medium, explant shoot length ranged from 2.7 to 4.6 mm across different hormone combinations. Average shoot length ranged from 2.5 to 4.5 mm along the passages. In hormone combination BAP 1.0: IAA 0.5, a maximum shoot length of 4.6 mm was observed. General trend was increase in shoot length along the passages on WPM medium.

In rooting experiment, per cent survival of the shoots varied from 72.7 per cent to 83.3 per cent. Root initiation ranged from 18.2 per cent to 66.7 per cent. Average explant survival across hormone treatments was 77.6 per cent and root initiation was 44.4 per cent. Maximum shoot survival of 83.3 per cent was observed with IBA 1.0 ppm. Highest root induction of 66.7 per cent was observed with

IBA 1.5 ppm (Table 1.4 and Plate 1.3). Further increase in the IBA concentration to 2.5 ppm drastically reduced root initiation. There was no clear-cut trend in shoot survival and root induction.

During the last 15 years or so, a number of investigations have been carried out on *in-vitro* propagation of seabuckthorn (Burdasov and Sviridenko, 1988), Montpetitand Lalonde (1988), Nikov and Tretyakova, (1993), Yao (1994) and Guo Chunhua *et al.* (2000). Recently, Lummerding (2001) in Canada, has also worked on the development of micropropagation protocol of seabuckthorn. Our study has shown very promising results for the successful standardization of protocol for the seabuckthorn, which grows widely in Himalayas.

Table 1.4: Effect of IBA on Shoot Survival and Root Induction

Treatment	***Per cent Survival***	***Per cent Root Initiation***
IBA 0.5 ppm	81.8	45.5
IBA 1.0 ppm	83.3	33.3
IBA 1.5 ppm	75	66.7
IBA 2.0 ppm	75	58.3
IBA 2.5 ppm	72.7	18.2
Average	77.6 per cent	44.4 per cent

CONCLUSIONS

1. Explant surface sterilization with Teepol (0.1 per cent), 250 mg Tetracycline, Ethenol (70 per cent), $HgCl_2$(0.1 per cent) was effective to initiate >90 per cent aseptic cultures from active and dormant buds of seabuckthorn.

2. Explant treatment with citric acid + ascorbic acid solution (1500 ppm), followed by cold treatment 4-5°C for 5-6 days, reduced phenolics accumulation.

3. Media pH of 5.7-5.8 was found to be optimal for reducing explant phenolics accumulation.

4. Preculturing of cold treated explant on plain agar supplemented with 3 per cent Sucrose and 100 ppm Inositol, pH 5.8 for 2 weeks, before transferring explant on to plant tissue culture media completely controlled phenolics accumulation and helped in breaking dormancy of more than 90 per cent of dormant buds.

5. WPM medium was found to be better in controlling explant vitrification as well as vitrification level (42.2 per cent vitrification) compared to MS (45 per cent vitrification) and ½ MS (68.3 per cent vitrification).

6. Explant survival was found to be highest (75 per cent) on WPM medium in comparison to MS (50 per cent), and ½ MS (70 per cent).

7. Comparison of media-hormone interactions revealed that in all three group combination of hormones (BAP: NAA, BAP: IAA, BAP: IBA) the explant survival was highest in WPM and lowest in MS.

8. Along the successive passages, per cent multiple shoots, shoots/explant increased on WPM while on MS and ½ MS it decreased.

9. Multiple shoot per cent, shoot/explant was found to be highest on WPM medium.

10. Hormone combinations BAP 1.0: IAA 0.5 (60 per cent) and BAP 0.3: NAA 0.2 (66.7 per cent), led to development of multiple shoots in maximum number of cultures on WPM medium

11. Hormone combinations BAP 1.0: IAA 0.5 (14.6 shoots/explant) and BAP 0.3: NAA 0.2 (5 shoots/explant), led to development of maximum number of shoots/explant on WPM medium.

12. There was increase in shoot length along the passages on WPM medium where as it decreased along the passages on ½ MS medium.

13. On WPM medium hormone combination BAP 1.0: IAA 0.5 led to maximum increase in shoot length (4.6 mm).

14. Highest root induction was observed with IBA 1.5 ppm on WPM rooting medium (66.7 per cent).

15. Maximum shoot survival on the WPM rooting medium was observed with IBA 1.0 ppm (83.3 percent).

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