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RESEARCH ARTICLE

# Micropropagation of Seabuckthorn (*Hippophae rhamnoides* ssp. *turkestanica*)

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**Abstract**: Studies on the development of protocol was conducted on the Lahaul form of *H. rhamnoides* ssp. *turkestanica*), the most promising form of the Himalayan seabuckthorn. With active bud explants of seabuckthorn, more than 95% contamination free cultures were established through surface sterilization scheme of 0.1% detergent (2 Hours), Tetracycline (2 Hours), 70% EtOH (4 Min) and 0.1 % $HgCl_2$ (6 Min), whereas with dormant buds 0.1% detergent (5 Hours), Tetracycline (over night), 70% EtOH (15 Min) and 0.1 % $HgCl_2$ (18 Min). There was increase in overall explant survival from MS through ½MS to WPM, and decrease in explant vitrification from ½MS 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%), closely followed by ½MS (80%) and lowest on MS medium. Overall on MS medium, % 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% of the cultures, followed by maximum of 1.1 shoots/explant in BAP 0.2: IBA 0.01 in 40% of the cultures during first stage of culturing passage (PI). On WPM medium, in 60% 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% cultures having maximum of 5 shoot/explant in BAP 0.3: NAA 0.2 ppm combination during PI. Overall there was increase in % 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 % multiple shoot development and shoot/explant across different culture media from MS through ½MS to WPM along successive passages. Maximum shoot survival of 83.3% was observed with IBA 1.0 ppm. Highest root induction of 66.7% was observed with IBA 1.5 ppm. Among different culture media tested with various growth hormone combinations, WPM medium with 3% sucrose, was found to be suitable for the induction of multiple shoots, with hormone combination of BAP 1.0: IAA0.5 ppm and WPM with 2% 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; shoot tips; ms; wpm; vitrification; multiple shoots formation; rooting.

**Abbreviations**: MS: Murashige-Skoog, WPM: Woody plant medium, IAA: Indole acetic acid, NAA: Naphthalene acetic acid, IBA: Indole butyric acid, EtOH: Ethyl alcohol, BAP: Benzylaminopurine, PI: First stage of culturing passage, ppm: Part per million.

## Introduction

Seabuckthorn (*H. rhamnoides* ssp. *turkestanica*) 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 the control of soil erosion and improvement. It also has been used in soil reclamation for its ability to fix nitrogen and conserve other essential nutrients (Li and 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

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-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 study aims to standardize the protocol of Indian seabuckthorn (*Hippophae rhamnoides* ssp. *turkestanica)*, which grows in high altitude Indian Himalayas.

## Materials 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 were surface sterilized using 0.1% detergent solution of teepol in 1500 ppm solution of ascorbic acid and citric acid each for 2 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 (Nilov and Tretyakova, 1993). 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% 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% $HgCl_2$ 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 were surface sterilized using 0.1% 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% EtOH (Ethyl alcohol) 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% $HgCl_2$ 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% plain agar, pH 5.8, supplemented with 100 ppm Inositol and 3% 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% agar, pH 5.8, supplemented with 100 ppm Inositol and 3% 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% agar, pH 5.8, supplemented with 100 ppm Inositol and 2% sucrose. Cultures were kept under 16/8 hours photoperiod at 18˚C for 4 to 6 weeks.

## Results and discussion

*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.

**Table 1:** Effect of pH on phenolic accumulation in explants of seabuckthorn.

| 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% 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% of the dormant buds. These measures are summarized in Table 2.

**Table 2:** Remedial measures to over come phenolic accumulation.

| S.No. | Treatment | Concentration/ Quantity | Duration |
|---|---|---|---|
| 1 | Ascorbic acid + Citric acid w/v in sterile DDW | 1500 ppm each | 60 minutes |
| 2 | Low temperature | 4-5 ˚C | 5-6 days |
| 3 | Pre culture of explant on plain Agar supplemented with sucrose and inositol | 3% 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 & BAP 0.5: IAA 1.0 resulted in highest level of vitrification in up to 20% of the cultures.

Overall, 45% of the cultures had varying levels of vitrification and 55% cultures were normal. The explant survival rate varied from 20-80%. Combinations BAP 1.0: IAA 0.5, BAP 0.05: IAA 2.0 & BAP 0.1: IBA 0.5 had highest

mortality with up to 80% dead cultures. The overall explant survival was 50%.

*On ½MS medium*

On ½MS 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% of the cultures had varying levels of vitrification and 31.7% 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 & BAP 0.1: IBA 0.5 had highest level of vitrification ranging from 20-40% of the cultures. The explant survival rate varied from 40-100%. Combinations BAP 0.2: IBA 0.01 and BAP 0.3: NAA 0.1 had 100% explant survival. The overall explant survival was 70%.

*On WPM*

In 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 & BAP 0.01: IBA 0.2 had highest levels of vitrification ranging from 12.5% to 62.5% of the cultures. Overall, 42.4% of the cultures had varying levels of vitrification and 57.6% cultures were normal. The explant survival rate varied from 62.5-100%. Combination BAP 0.5: IBA 0.1 had 100% explant survival. The overall explant survival was 75%.

General trend was an increase in overall explant survival from MS through ½MS to WPM, decrease in explant vitrification from ½MS 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%), closely followed by ½MS (80%) 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, % multiple shoot ranged from 0 to 40% across the different hormone combinations (BAP + NAA, BAP + IAA, BAP + IBA). Highest multiple shoots in 40% of the cultures were observed in BAP 0.5: IAA 1.0 combination. Overall 11.7% 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% across different hormone combinations. In hormone combination BAP 0.2: IBA 0.01, callusing was observed in 20% of the cultures. Overall 1.7% of the P 0 cultures had callusing.

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

*On ½MS medium*

On ½MS medium, during P 0 passage, % multiple shoot ranged from 0 to 60% across the different hormone combinations (BAP + NAA, BAP + IAA, BAP + IBA). Maximum multiple shooting in 60% cultures was observed in combinations BAP 0.2: IBA 0.01 & BAP 0.1: IBA 0.5. Overall 26.7% of the P 0 cultures had multiple shoots ranging from 0 to 1.4 shoots/explant. In 60% of the cultures, shoots/explant of a maximum 1.4 shoots/explant was observed in combination BAP 0.2: IBA 0.01 (Plates 1 a, b and c). Average shoots/explant was 0.4. In P 0 cultures callusing ranged from 0 to 60 % across different hormone combinations. Maximum of 60 % callusing was observed in hormone combination BAP 0.1:

IBA 0.5. Overall 15.0% of the P 0 cultures had callusing.

In P I passage cultures, multiple shoots ranged from 0-60% across different hormone combinations. Overall, 21.7% of the P I 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% cultures. In combination BAP 0.2: IBA 0.01, 40% of the cultures had a maximum of 1.1 shoots/explant. Average shoots/explant was 0.4. There was no callusing.

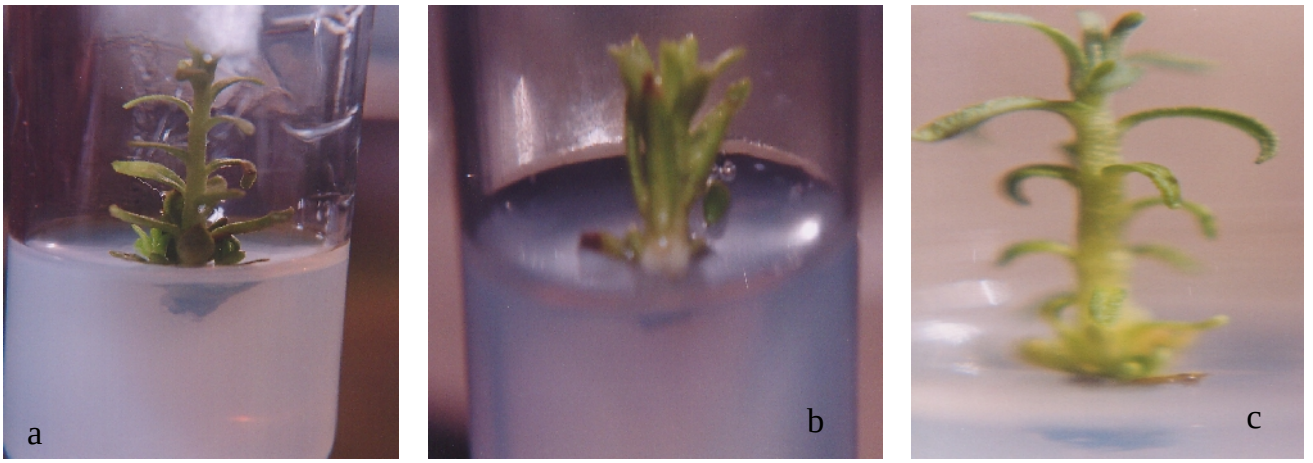


**Plate 1a & b:** Development of multiple shoots; **Plate 1 c:** Elongation of subcultured shoot on ½MS

In P II passage cultures, multiple shoots ranged from 0-40% across different hormone combinations. Overall, 17.5% 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: IAA 0.5, multiple shooting was observed in 40% of the cultures. There was 0-20% callusing. Overall 1.7% of the cultures had callusing. In combination BAP 0.3: NAA 0.2, callusing was observed in 20% of the cultures.

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

*On WPM medium*

On WPM medium, during P 0 passage, % multiple shoots ranged from 0 to 60% across the different hormone combinations (BAP + NAA, BAP + IAA, BAP + IBA). In combination: BAP 0.2: NAA 0.05 ppm, 60% of the cultures developed multiple shoots**.** Overall 6% 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 I passage, multiple shoots ranged from 0-66.7% across different hormone combinations. Overall, 17.7% of the P I cultures had multiple shoots. In hormone combinations BAP 0.3: NAA 0.2, multiple shooting was observed in 66.7% cultures. Shoots/explant ranged from 0-14.6 shoots/explant. Average shoots/explant was 1.7. In 60% 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% cultures having maximum of 5 shoot/explant in combination BAP 0.3: NAA 0.2 ppm. Callusing ranged from 0 to 60% across different hormone combinations. Overall in 13.3% cultures, callusing was observed. In BAP 1.0: IAA 0.5 combination, 60% cultures had callusing.

In P II passage cultures, multiple shoots ranged from 0-80% across different hormone combinations. Overall, 15% of the P II cultures had multiple shoots. In hormone combinations BAP 1.0: IAA 0.5 ppm, multiple shooting was

observed in 80% of the cultures with maximum of 6.5 shoots/explant, which was followed by 30% cultures in combination BAP 0.3: NAA 0.2 ppm with maximum of 1.7 shoots/explant (Plates 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% callusing. Average callusing was 9.2%. In combinations BAP 1.0: IAA 0.5, callusing was observed in 60% of the cultures.

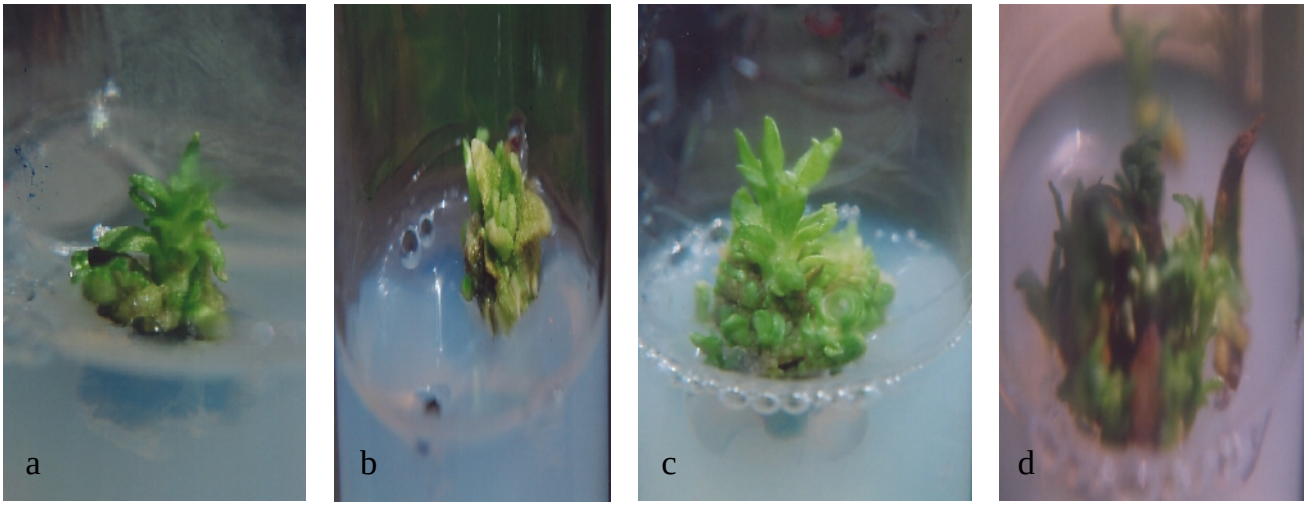


**Plate 2a & b:** Development of multiple shoots with BAP 0.3 + NAA 0.2 ppm on WPM.

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%. Overall, 42.2% of the P III cultures had multiple shoots. In hormone combinations BAP 1.0: IAA 0.5, multiple shooting was observed in 80% 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% of the cultures. In combination BAP 0.3: NAA 0.2, callusing was observed in 26.7% of the cultures.

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

Overall Trend was increase in % multiple shooting, shoots/explant and callusing along successive passages on WPM Medium. Overall trend with WPM was decrease in % 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% cultures developed multiple shoots in WPM medium (P II passage), followed by 40% in ½MS (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% cultures developed multiple shoots in WPM medium (P II & P III passages), followed by 60% cultures in WPM medium (P I passage), which was followed by 40% cultures in ½MS (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% cultures both in MS (P 0 passage) and WPM (P II passage) developed multiple shoots, followed by 20% cultures in WPM (P III passage) and 10 % cultures in ½MS (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 I passage with BAP 0.3:

NAA 0.2 & BAP 1.0: IAA 0.5 combinations. With BAP 0.5: IAA 1.0, the multiple shoots started developing both in WPM and ½MS from P II passage.

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

Comparison of development of shoot/explant on different culture media showed that, during successive passages with BAP 0.3: NAA 0.2 hormone combination in WPM medium, a maximum of 5 shoots/explant developed (P I passage), followed by 1.7 shoot/explant (P II), 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 I 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 3).

On the other hand in BAP 0.5: IAA 1.0 combination with MS, ½MS 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 ½MS to WPM along successive passages.

On ½MS 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 ½MS 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.

**Table 3**: Multiple shoots/explant during successive passage on different culture media.

| **Treatment** | **MS** | | **½MS** | | | | **WPM** | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **P 0** | **P1** | **P 0** | **P I** | **P II** | **P III** | **P 0** | **P I** | **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 |

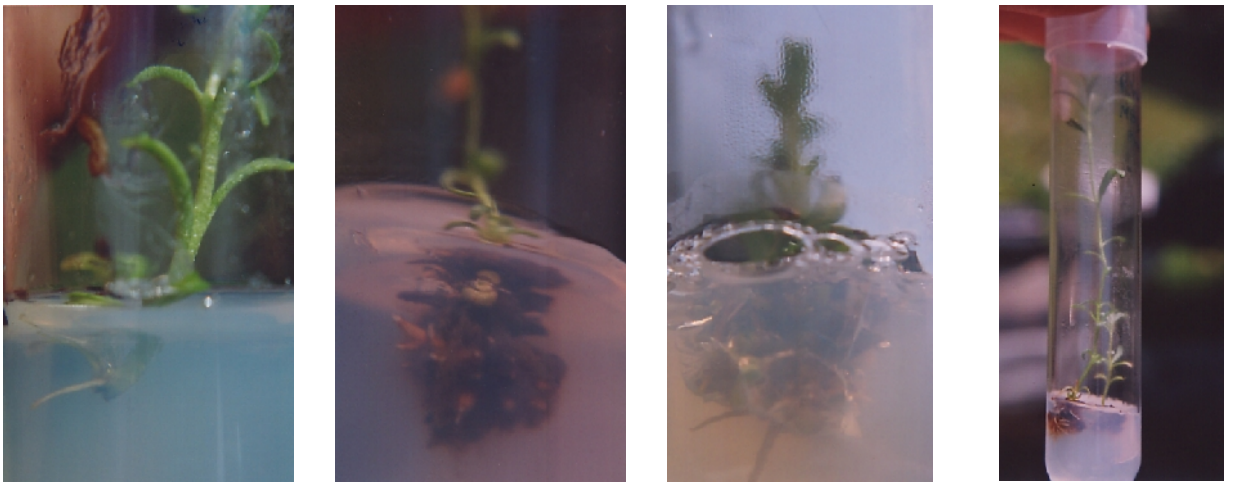

**Plate 3**: Root induction on WPM rooting medium with IBA 1.5 ppm.

**Table 4:** Effect of IBA on shoot survival and root induction

| Treatment | Survival (%) | 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% | 44.4% |
| CD (P<0.05) | 4.3 | 7.1 |

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

IBA 1.5 ppm (Table 4 and Plate 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), Montpetit and Lalonde (1988), Nikov and Tretyakova, (1993), Yao (1994) and Guo Chunhua *et al*. (2000). 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.

## Conclusion

WPM medium was found to be better in controlling explant vitrification as well as vitrification level (42.2% vitrification), as compared to MS (45% vitrification) and ½MS (68.3% vitrification). Explant survival was found to be highest (75%) on WPM medium in comparison to MS (50%), and ½MS (70%). 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. Along the successive passages, % multiple shoots, shoots/explant increased on WPM while on MS and ½MS it decreased. Multiple shoot %, shoot/explant was found to be highest on WPM medium. Hormone combinations BAP 1.0: IAA 0.5 (60%) & BAP 0.3: NAA 0.2 (66.7%), led to development of multiple shoots in maximum number of cultures on WPM medium. Hormone combinations BAP 1.0: IAA 0.5 (14.6 shoots/explant) & BAP 0.3: NAA 0.2 (5 shoots/explant), led to development of maximum number of shoots/explant on WPM medium. There was increase in shoot length along the passages on WPM medium where as it decreased along the passages on ½MS medium. On WPM medium hormone combination BAP 1.0: IAA 0.5 led to maximum increase in shoot length (4.6 mm). Highest root induction was observed with IBA 1.5 ppm on WPM rooting medium (66.7%). Maximum shoot survival on the WPM rooting medium was observed with IBA 1.0 ppm (83.3%).

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