

Effect of Mulching, Shading, Spacing and Cutting Thickness on Propagation of Seabuckthorn (*Hippophae rhamnoides* L.) by Cuttings

Phuntsog Dolkar¹, Phunchok Angmo¹, Diskit Dolkar¹, Bhuvnesh Kumar[#],
O.P. Chaurasia¹, and Tsering Stobdan^{*1}

¹DRDO-Defence Institute of High Altitude Research, Leh - 194 101, India

[#]DRDO-Defence Institute of Physiology and Allied Sciences, Delhi - 110 054, India

^{*}E-mail : ts_mbb@yahoo.com

ABSTRACT

The effect of plastic mulching, coloured shade netting, spacing and cutting thickness on rooting success and growth of Seabuckthorn (*Hippophae rhamnoides* L.) through hard wood cuttings is addressed. Use of silver black plastic mulching film resulted in 10 per cent higher rooting success and significant plant growth. Suppression of weed emergence by the plastic mulch resulted in 75.8 per cent time saving in manual weeding by farm workers. No significant gender difference in rooting success was observed. Reduction in light intensity by 66 per cent using green shade net resulted in significant reduction in rooting and growth of nursery plants. Three different spacing between cuttings did not show significant difference in rooting and growth related parameters suggesting that cuttings can be planted denser (3"×3") under mulching to get higher number of nursery stock per unit area. Cutting thickness showed significant effect on rooting success. Highest rooting percentage was observed in pencil thickness cuttings (7.5 ± 1.6 mm dia) followed by cuttings with 2.9 ± 0.8 mm and 11.3 ± 1.7 mm basal diameter. The result of the present study could facilitate establishment of a vegetative propagation method wherein faster growth and larger number of cuttings can be propagated with higher rooting success rate.

Keyword: Cuttings; Seabuckthorn; Himalaya; Nursery; Vegetative propagation

1. INTRODUCTION

Seabuckthorn (*Hippophae rhamnoides* L.) is an ecologically and economically important temperate shrub. The species is dioecious and wind pollinated. Every part of Seabuckthorn (SBT) is being used in the Himalayan region for variety of purposes. There are over a hundred popular SBT-based formulations reported in various pharmacopoeias of *Sowa Rigpa* (Tibetan medicinal system)¹. The shrub serves as a storehouse for researchers in the field of pharmaceutical, nutraceutical, cosmetic, biotechnology and environmental sciences². Scientific advancement in medicinal and therapeutic potential of the species has recently been reviewed³⁻⁵.

Recent interest in the vegetative propagation of SBT arises primarily because there is increasing demand for SBT nursery for plantation. SBT plantation is looked upon as a potential means for sustainable development particularly in Himalayan region. Hardwood cuttings are more often used in propagation of deciduous woody plants as one of the easiest and cheapest methods of vegetative propagation. Typically, they require less or no special equipment during rooting, which can be readily performed in nurseries⁶. Vegetative propagation through pencil thickness hardwood cutting is the method of choice for propagation of the species. Currently, there is a body of information concerning the propagation of SBT through pencil thickness (5 mm - 10 mm diameter) hardwood stem cuttings⁷⁻¹¹.

Propagation through one year old growth stem cutting (2.9±0.8 mm diameter), which is less than half the thickness of conventional pencil thickness hardwood cutting, has recently been studied to obtain larger number of cuttings per plant¹². However, the influence of mulching, shading, spacing and cutting thickness on rooting success and growth has received almost no attention. Therefore, the objective of the present study was to study the effect of plastic mulching, coloured shade net, spacing and cutting thickness on rooting success and growth of SBT in nurseries. The present result could facilitate establishment of a vegetative propagation method wherein faster growth and larger number of cuttings can be propagated with higher rooting success rate.

2. MATERIALS AND METHODS

1.1 Study Site

The study was conducted in 2014 and 2015 at Defence Institute of High Altitude Research (trans-Himalaya, India, 34°08.2'N; 77°34.3'E, elevation 3350 m) on a flat site with direct sunshine. The mean minimum and maximum temperature recorded daily during cropping season (April-October) in 2015 was 5.8±5.2 °C and 18.8±5.4 °C, respectively, while the mean minimum and maximum relative humidity was 22.1±2.0 % and 28.3±2.7 %, respectively.

2.2 Plant Materials

H. rhamnoides subsp. *turkestanica* stem cuttings of 15.3±1.2 cm in length were taken from female plants in first

week of April from natural SBT growing in trans-Himalayan Ladakh region. Thorns on each cutting were removed. The base of cuttings were cut and soaked in tap water for 24 h followed by 500 ppm indole-3-butyric acid (IBA) treatment for 1 min. IBA solution was prepared in 50 % isopropanol. They were planted in nursery bed at spacing of 6"×6" gap between cutting to cutting and row to row, except in spacing trial. Each plot (4.57 m²) received 3.5 kg farm yard manure at time of field preparation. Irrigation was provided by flooding system at weekly interval.

2.3 Mulching, Shading, Spacing, Cutting Thickness

Silver black plastic film (30 micron) was used for mulching with black surface facing the sun. The result was compared with cuttings under non-mulched condition. The experiment was designed as complete randomised block with three replicates of 126 female cuttings each. A subsidiary set of three plots were established in an adjacent section with cuttings from male plant to study the effect of gender on rooting. Weed emergence and time consumed in manual weeding in mulched and non-mulched plots was calculated in terms of fresh weight of weed (kg/plot) and time devoted by a single farm worker in weeding (min/plot), respectively. To study the effect of shading, three replicates of 126 cuttings each were shaded with a green shade net used in nurseries to give approximately 66 % reduction of sunlight. The nets were fixed on quonset-shaped structures consisting of metal pipes framework with 2'6" height at the centre. Rooting success and plant growth was compared with cuttings grown in open field conditions. The light intensity measured with Datalogging light meter (HD450, Extech Instruments) at noon in open field was 131193 ± 43574 lux and that of shade net conditions was 44353 ± 17335 lux. Therefore, the shade net used reduced light intensity by 66.2 per cent. Three nursery spacing treatment (6"×6", 3"×3", triangular system i.e additional one cutting in the centre of 6"×6") was studied. The experiment was designed as complete randomised block with three replicates of 126-480 cuttings each depending on spacing. To study the effect of cutting thickness, three cuttings thickness with basal diameter of 11.3±1.7 mm, 7.5±1.7 mm and 2.9±0.8 mm were taken. The experiment was designed as complete randomised block with three cutting thickness with three replicates of 126 cuttings each in open field condition. Rooting success and plant growth parameters were scored in mid-October. Ten rooted cuttings from each replicate were sampled from different treatments. After washing the rooted cuttings, root and shoot growth related parameters were recorded.

2.4 Statistical Analysis

One-way ANOVA was performed with the help of 2-sided Tukey's HSD at $P \leq 0.05$. All statistical analysis was performed using SPSS for Windows 17.0 version.

3. RESULTS AND DISCUSSION

3.1 Effect of Mulching

Black plastic mulching significantly increased rooting percentage and growth of nursery plants (Table 1). Approximately 10 per cent higher rooting was observed in mulched as compared to non-mulched in open field conditions

suggesting that mulching provide a suitable condition for higher rooting success for SBT (Fig. 1). However, mulching does not significantly increase number of shoot per cutting. Higher rooting success and growth of nursery plants in mulched condition may be attributed to several factors. In the present study, average monthly soil temperature recorded at noon at 10 cm below soil surface in mulched soil was 1.6 °C - 3.6 °C higher than non-mulched soil. Therefore, higher soil temperature particularly in the early growth of the plant is beneficial in cold climatic region such as the trans-Himalayan Ladakh region. Since irrigation intervals were similar for both treatments, it is possible that lower evaporation rates beneath the plastic mulch also contributed to higher growth. Suppression of weeds is yet another important factor contributing to higher plant growth in mulched condition. Mulching significantly reduce the emergence of weed. Fresh weight of weeds recorded in mulched soil was 2.0±0.7 kg/plot as compared to 6.4±2.4 kg/plot in non-mulched soil. Suppression of weed emergence by the plastic mulch resulted in 75.8% time saving in manual weeding by farm workers (mulched: 18.7±2.5 min/plot; non-mulched: 77.3±2.6 min/plot). No significant gender difference in rooting success was observed in mulched condition (Table 1). However, significantly higher shoot length, number of primary root and basal diameter of secondary root was observed in male as compared to female plants. In contrast, Dhyani¹³, *et al.* reported higher rooting success and number of roots per cuttings in female cuttings than in male donor plants in *H. salicifolia*. More studies are therefore needed to determine gender effect on rooting success.



Figure 1. One year old rooted Seabuckthorn cutting.

Table 1. Effect of mulching, shading, gender, spacing and cutting thickness on rooting percentage and growth of Seabuckthorn cuttings in nursery

| Treatment | Rooting % | Number of shoot per cutting | Average shoot length (cm) | Number of leaf per shoot | Number of primary root per cutting | Number of secondary root per cutting | Length of longest primary root (mm) | Length of longest secondary root (mm) | Diameter of basal end of primary root (mm) | Diameter of basal end of secondary root (mm) |
|---|------------------------|-----------------------------|---------------------------|--------------------------|------------------------------------|--------------------------------------|-------------------------------------|---------------------------------------|--|--|
| A. Mulching, shading and gender of donor plant | | | | | | | | | | |
| Mulch (female) | 84.0±5.3 ^b | 7.2±2.0 ^a | 20.3±3.8 ^{bc} | 40.1±7.3 ^c | 8.3±3.3 ^{ab} | 60.8±12.2 ^a | 63.3±27.4 ^b | 25.8±3.4 ^b | 3.5±0.6 ^a | 1.6±0.5 ^{ab} |
| Non-mulch | 75.7±4.1 ^{ab} | 5.7±0.6 ^a | 13.5±1.7 ^{ab} | 28.0±6.1 ^{ab} | 6.0±1.7 ^{ab} | 38.3±11.6 ^{abc} | 34.8±3.6 ^{ab} | 14.1±6.6 ^{ab} | 2.2±0.3 ^a | 0.8±0.1 ^a |
| Mulch + shade | 78.3±0.6 ^{ab} | 5.8±1.2 ^a | 15.6±2.1 ^{ab} | 34.8±6.1 ^{bc} | 7.5±2.1 ^{ab} | 35.0±9.8 ^{ab} | 32.0±18.2 ^{ab} | 10.8±5.4 ^a | 2.1±0.6 ^a | 0.7±0.3 ^a |
| Non-mulch + shade | 72.7±1.1 ^a | 4.0±1.8 ^a | 9.3±6.5 ^a | 17.7±6.6 ^a | 4.0±1.8 ^a | 23.5±14.9 ^a | 16.7±10.2 ^a | 6.4±2.4 ^a | 2.2±1.4 ^a | 0.8±0.4 ^a |
| Mulch (male) | 84.7±2.5 ^b | 5.3±2.2 ^a | 28.1±7.5 ^c | 42.9±2.4 ^c | 9.0±1.3 ^b | 55.7±16.3 ^{bc} | 63.1±16.2 ^b | 25.2±11.6 ^b | 3.5±0.4 ^a | 2.1±0.6 ^b |
| B. Spacing between plant to plant and row to row (mulched) | | | | | | | | | | |
| 6"×6" | 83.5±1.1 ^a | 6.7±1.0 ^a | 23.9±4.9 ^a | 43.0±12.1 ^a | 8.2±3.8 ^a | 62.5±8.4 ^a | 55.6±4.1 ^a | 22.7±2.8 ^{ab} | 4.1±0.6 ^a | 1.4±0.3 ^a |
| 3"×3" | 84.0±5.3 ^a | 7.2±2.0 ^a | 20.3±3.8 ^a | 40.1±7.3 ^a | 8.3±3.3 ^a | 60.8±12.2 ^a | 63.3±27.4 ^a | 25.8±3.4 ^{ab} | 3.5±0.6 ^a | 1.6±0.5 ^a |
| *Triangular | 84.0±1.2 ^a | 5.8±0.8 ^a | 19.0±3.1 ^a | 40.5±4.8 ^a | 7.5±1.8 ^a | 46.2±13.2 ^a | 66.1±20.2 ^a | 12.9±5.4 ^a | 3.7±0.4 ^a | 1.3±0.5 ^a |
| C. Cutting thickness: > Pencil thickness (11.32±1.7 mm); Pencil thickness (7.45±1.6 mm); <Pencil thickness (2.9±0.8 mm) (non-mulched) | | | | | | | | | | |
| > Pencil thickness | 50.4±6.6 ^a | 6.7±2.1 ^a | 13.2±6.8 ^a | 27.5±9.5 ^a | 7.3±3.5 ^a | 36.3±2.1 ^a | 50.6±21.3 ^a | 10.7±3.0 ^a | 2.2±0.3 ^a | 0.8±0.2 ^a |
| Pencil thickness | 75.7±4.1 ^{ab} | 5.7±0.6 ^a | 13.5±1.6 ^a | 28.0±6.1 ^a | 6.0±1.7 ^a | 38.3±11.6 ^a | 34.8±3.6 ^a | 14.1±6.6 ^a | 2.2±0.3 ^a | 0.8±0.1 ^a |
| <Pencil thickness | 65.0±1.7 ^{ab} | 5.3±0.6 ^a | 12.4±0.5 ^a | 25.5±2.1 ^a | 6.3±0.6 ^a | 33.7±2.1 ^a | 37.3±1.2 ^a | 19.8±3.4 ^a | 2.4±0.4 ^a | 0.8±0.2 ^a |

Values represented as mean±SD of 30 cuttings; for each column, different lowercase letters within a column in each treatment (A, B and C) indicate significantly different at $P \leq 0.05$, as measured by 2-sided Tukey's HSD; *Triangular: additional one cutting in the centre of 6"×6"

3.2 Effect of Shading

Effect of shading on the rooting and growth of SBT cuttings has not been reported earlier. If nurseries can be raised under low light without impairing their quality and rooting success, a considerable amount of water will be saved. However, in the present study, reduction in light intensity by 66 per cent using green shade net was found to significantly reduce the rooting success, shoot length, number of leaf, length and number of roots in mulched condition (Table 1). This agrees with previous results in shade-intolerant pioneer *Pinus halepensis* where shading treatment produced low quality seedlings with poor root development¹⁴. Therefore, more studies are needed using different coloured shade nets of different light intensity to optimally exploit the benefits of nursery shading materials in SBT.

3.3 Effect of Spacing

Study on relationship between spacing and growth of nursery plant is important for nursery managers. If more number of nursery plants can be raised per unit area without affecting the nursery quality, it results in higher income. Results in the present study on three different spacing between cuttings under mulching did not show significant difference in rooting success and growth related parameters in one year old nurseries (Table 1). Therefore, SBT cuttings can be planted denser (3"×3") to

get higher number of nursery stock per unit area. Wider spacing between seedlings is reported to result in higher nutrient uptake and better outplanting performance in two year old nursery stock of Douglas-fir (*Pseudotsuga menziesii*) and Sitka spruce (*Picea sitchensis*)¹⁵.

3.4 Effect of Cutting Thickness

Cutting thickness showed significant effect on rooting success but did not affect growth related parameters (Table 1). Highest rooting percentage in open field condition was observed in pencil thickness cuttings (7.5±1.7 mm diameter) followed by cuttings with less than half the pencil thickness (2.9±0.8 mm diameter) and those with more than pencil thickness diameter (11.3±1.7 mm). Therefore, pencil thickness cutting is most suitable for propagation of SBT in open field conditions. Higher rooting percentage in these cuttings is in agreement with studies by Exadaktylou⁶, *et al.* on cherry rootstock. The authors reported that cherry rootstock cutting with diameters of 6-8 mm and 9-11 mm showed the highest rooting percentage, whereas no rooting was observed for those with 12-14 mm diameter.

4. CONCLUSIONS

Use of black plastic mulching resulted in 10 per cent higher rooting success and 75.8 per cent time saving in

manual weeding. Reduction in light intensity by 66 per cent using shade net is not advisable for nursery managers since it resulted in significant reduction in rooting success and growth of nursery plants. SBT can be planted denser (3"×3") under mulching condition to get higher number of nursery stock per unit area. Pencil thickness (7.5±1.7 mm diameter) cutting is most suitable for propagation of SBT in open field conditions. However, cutting thickness with 2.9±0.8 mm and 11.3±1.7 mm basal diameter can also be used for propagation with high success rate. The result of the present study could facilitate establishment of a vegetative propagation method wherein faster growth and larger number of cuttings can be propagated with high success rate.

Conflict of Interest: None

REFERENCES

1. Stobdan, T.; Targais, K.; Lamo, D. & Srivastava, R.B. Judicious use of natural resources: a case study of traditional uses of Seabuckthorn (*Hippophae rhamnoides* L.) in trans-Himalayan Ladakh, India. *Nat. Acad. Sci. Lett.*, 2013, **36**, 609-13.
doi:10.1007/s40009-013-0177-4
2. Stobdan, T., Angchuk, D. & Singh, S.B. Seabuckthorn: An emerging storehouse for researchers in India. *Current Science*, 2008, **94**, 1236-7.
3. Geetha, S. & Gupta, A. Medicinal and therapeutic potential of Sea buckthorn (*Hippophae rhamnoides* L.). *J. Ethnopharmacol.*, 2011, **138**, 268-78.
doi: 10.1016/j.jep.2011.09.024
4. Kanayama, Y.; Kato, K.; Stobdan, T.; Galitsyn, G.G.; Kochetov, A.V. & Kanahama, K. Research progress on the medicinal and nutritional properties of seabuckthorn (*Hippophae rhamnoides*) – a review. *J. Hortic. Sci. Biotech.*, 2012, **87**, 203-10.
doi: 10.1080/14620316.2012.11512853
5. Stobdan, T.; Korekar, G. & Srivastava, R.B. Nutritional attributes and health application of seabuckthorn (*Hippophae rhamnoides* L.) – a review. *Curr. Nutr. Food Sci.*, 2013, **9**, 151-65.
doi: 10.2174/1573401311309020008
6. Exadaktylou, E.; Thomidis, T.; Grout, B.; Zakyntinos, G. & Tsipouridis, C. Methods to improve the rooting of hardwood cuttings of the 'Gisela 5' cherry rootstock. *HortTechnol.*, 2009, **19**, 254-59.
7. Lu-Rongsen. Seabuckthorn- a multipurpose plant for fragile mountains. Occasional Paper No. 20, International Centre for Integrated Mountain Development, Kathmandu, Nepal, 1992
8. Singh, V. Rooting rates of hardwood cuttings of seabuckthorn. *J. Tree Sci.*, 1995; **14**, 87-8.
9. Dwivedi, S.K.; Paljor, E.; Attrey, D.P. & Singh, B. Propagation of common seabuckthorn (*Hippophae rhamnoides*) through hard wood cutting in Ladakh. *Indian J. Agric. Sci.*, 2002, **72**, 228-9.
10. Rao, V.K.; Yadav, V.K.; Sharma, S.K. & Sah, V.K. Effect of season and plant bio-regulators on hardwood cutting of seabuckthorn (*Hippophae salicifolia*) under Garhwal Himalayas. *Indian J. Agric. Sci.*, 2010, **80**, 910-12.
11. Raj, X.J.; Ballabh, B.; Murugan, M.P.; Dhar, P.; Tayade, A.B.; Warghat, A.R.; Chaurasia, O.P. & Srivastava, R.B. Effect of auxin on adventitious rooting from hardwood cuttings of *Hippophae rhamnoides* under Ladakh Himalayas. *Indian Forester*, 2013, **139**, 228-31.
12. Dolkar, P.; Dolkar, D.; Angmo, S.; Srivastava, R.B. & Stobdan, T. An improved method for propagation of Seabuckthorn (*Hippophae rhamnoides* L.) by cuttings. *Natl. Acad. Sci. Lett.*, 2016, **39**, 323-6.
doi: 10.1007/s40009-016-0489-2
13. Dhyani, D.; Maikhuri, R.K. & Dhyani, S. Effect of auxin treatments on male and female cuttings of *Hippophae salicifolia*. *Afr. J. Biotechnol.*, 2012, **11**, 15712-8.
14. Puértolas, J.; Benito, L.F. & Peñuelas, J.L. Effects of nursery shading on seedling quality and post-planting performance in two Mediterranean species with contrasting shade tolerance. *New Forest*, 2009, **38**, 295-308.
doi:10.1007/s11056-009-9148-5
15. Van den Driessche, R. Relationship between spacing and nitrogen fertilization of seedlings in the nursery, seedling mineral nutrition, and outplanting performance. *Can. J. For. Res.*, 1984, **14**, 431-6.
doi: 10.1139/x84-076

CONTRIBUTORS

Ms Phuntsog Dolkar, received her MSc (Biotechnology) from Barkatullah University. Currently working as a Senior Research Fellow and pursuing her PhD in the Plant Science Division, DRDO-Defence Institute of High Altitude Research, Leh. She is currently working on a project entitled 'Gender differences in antioxidant properties, phenotypic plasticity and freeze tolerance in Seabuckthorn (*Hippophae rhamnoides* L.) along an altitudinal gradient in trans-Himalayan Ladakh, India'. She conducted the experiments and contributed towards literature collection, data analysis and manuscript preparation.

Ms Phunchok Angmo, received MSc (Botany) from Panjab University. Currently working as a Senior Research Fellow and pursuing her PhD in the Plant Science Division, DRDO-Defence Institute of High Altitude Research, Leh. She currently works on a project entitled 'Quality and yield of vegetables under different greenhouse structures in trans-Himalayan Ladakh, India'. She contributed towards literature collection, data analysis and manuscript preparation.

Ms Diskit Dolkar, received MSc (Botany) from Barkatullah University. Currently working as a Senior Research Fellow and pursuing her PhD in the Plant Science Division, DRDO-Defence Institute of High Altitude Research, Leh. She is currently works on a project entitled 'Exploiting plant growth promoting rhizobacteria for enhanced crop productivity in Indian trans-Himalaya'. She contributed towards literature collection and data analysis.

Dr Bhuvnesh Kumar, received his BVSc & AH (Veterinary Sciences), MVSc and PhD in Veterinary Medicine from G.B. Pant University of Agriculture and Technology, Pantnagar

(Uttarakhand), in 1982, 1984 and 1999, respectively. Currently working as a Director, DRDO-Defence Institute of Physiology and Allied Sciences, Delhi. He has vast experience of working in mountainous regions covering western, central and north east Himalayas.

He has contributed in experimental design and manuscript preparation.

Dr O.P. Chaurasia obtained his PhD (Botany) from Magadh University Bodh Gaya, Bihar, in 1992. Currently working as Scientist 'F' and Director, DRDO-Defence Institute of High Altitude Research, Leh. He has extensively surveyed trans-Himalayan belts of Ladakh and Lahaul-Spiti and documented

the fragile plant biodiversity and its ethnobotanical wealth. He contributed in experimental design and manuscript preparation.

Dr Tsering Stobdan received his PhD in Molecular Biology & Biotechnology from Indian Agricultural Research Institute, New Delhi. Currently working as Scientist 'E' and Head, Plant Science Division at DRDO-Defence Institute of High Altitude Research, Leh. He has 5 patents including one in USA, over 50 publications in journals, two monograph and 20 book chapters to his credit. He is the executive member of Seabuckthorn Association of India.

He designed the experiments and wrote the manuscript.