Defence Life Science Journal, Vol. 2, No. 1, January 2017, pp. 46-53, DOI : 10.14429/dlsj.2.11074

Seabuckthorn (*Hippophae rhamnoides* L.) in trans-Himalayan Ladakh, India

Tsering Stobdan*, Phuntsog Dolkar, O.P. Chaurasia, and Bhuvnesh Kumar

Defence Institute of High Altitude Research, Leh-Ladakh -194 101, India
**E-mail: ts_mbb@yahoo.com*

ABSTRACT

Seabuckthorn (SBT) is an ecologically and economically important plant species of trans-Himalayan Ladakh. Once considered a thorny menace, SBT is now being seen as a means for sustainable development of the trans-Himalayan region. A number of SBT-based antioxidant rich products are being developed that resulted in commercial spin-offs. SBT berry collection from natural habitat has become an important activity in the region since the year 2001. Currently the demand for SBT of the region exceeds the supply. Cultivation of SBT on 2500 ha would fetch a net income of Rs 491 crore (USD 72 million) annually. Anticipating the high demand for SBT studies are underway to meet the long term goal to introduce plantation of SBT on the available vast barren land. Potential of the lesser-known shrub has been recognised by several R&D organisations and a number of research institutes in India are working in this field. This article summarizes the advancement of research on SBT of trans-Himalayan Ladakh.

Keywords: Hippophae; Ladakh; Sea buckthorn; Sustainable development; Trans-Himalaya

1. INTRODUCTION

Seabuckthorn (*Hippophae rhamnoides* L.) is an ecologically and economically important thorny shrub, which is widely distributed in trans-Himalayan Ladakh region. The species is a wind pollinated dioecious shrub. The female plant bears red, orange or yellow berry on its two-year-old thorny branches. Seabuckthorn (SBT) berry is one of the most nutritious fruit having a lot of medicinal properties. Once considered a thorny menace, SBT is now being seen as a means for sustainable development of the trans-Himalayan region. Development of SBT-based products has resulted in commercial spin-offs to the extent that demand for its berry from the region now outstrip the supply; SBT berry which used to get Rs 8 per kg in the year 2001 is being sold for Rs 50 per kg in 2016.

In India SBT received much attention after Defence Institute of High Altitude Research (DIHAR), formerly Field Research Laboratory (FRL), had developed and perfected the technology for commercialised production of beverages from its highly acidic fruit[1]. Potential of the lesser-known shrub has been recognised by many R&D organisations. DRDO has pioneered the SBT research in India and started several R&D projects on SBT since early nineties. In recent years a number of research institutes in India are working on the lesser-known shrub. The shrub serves as a storehouse for researchers in the field of biotechnology, neutraceutical, pharmaceutical, cosmetic and environmental sciences[2]. This article summarizes the advancement of research on SBT of trans-Himalayan Ladakh. Ladakh region is characterised by extreme temperature variations, low precipitation mostly in the form of snow, high wind velocity, sparse plant density, thin atmosphere with high UV-radiation and fragile ecosystem. The average altitude of human habitation in Ladakh is over 3000 m amsl and the temperature drop up to -30 ºC in winter.

Received : 05 January 2017, Revised : 17 January 2017
Accepted : 19 January 2017, Online published : 28 March 2017

2.1 Geographical Distribution

The genus *Hippophae* comprises of seven species. *H. rhamnoides* and *H. tibetana* are found in Ladakh. Plants with characteristics of *H. salicifolia* are found in Suru valley in the region, which needs to be authenticated. *H. rhamnoides* is the predominant SBT species in trans-Himalayan Ladakh. It grows on a wide range of soil, ranging from highly acidic soil near hot springs to soil with high salt deposits[3]. The shrub is mainly distributed in Nubra, Indus and Suru valleys. Natural plantation is mainly distributed along Nubra, Shyok and Indus river. It is also found in Changthang region at an altitude of 15,400 ft amsl[1]. *H. tibetana* is found in selected pockets in Zanskar and Changthang area in the region[4]. Using satellite data, it is estimated that area under pure SBT is 7184 ha while the area under mixed SBT is 2083 ha in Ladakh[5]. In Nubra valley the shrub is estimated to be growing in 2876 ha[3]. Ladakh remains the major site for natural SBT resource with over 70 per cent of the total area (13,000 ha) under SBT in the country.

2.2 Traditional Uses

People living in trans-Himalayan region have developed mastery in judicious use of SBT resources. Every part of the plant is traditionally used for a variety of purposes be it for medicine, nutritional supplement, firewood, fencing, tree guard, wind break, building construction, religious rites, agricultural implements etc. Growing of SBT for fencing around agricultural fields is known to improve soil fertility. In Nubra valley, low fertile fields are often mixed with soil taken from densely growing SBT areas to improve soil fertility. *H. rhamnoides* is recorded as *Bar-sTar* in Tibetan medicine. There are over a hundred popular SBT-based formulations in various pharmacopoeias of *Sowa Rigpa* (Tibetan medicine), which is being practiced in Ladakh[6]. Despite the importance of SBT in the lives of people the age old knowledge of its usage is slowly declining due to opening of the region to the modern world and availability of alternatives from other parts of the world.

2.3 Genetic Diversity

High morphological diversity is observed in Ladakh (Figure 1). Singh[7], *et al.* studied genetic diversity of *H. rhamnoides* using RAPD markers. Seventeen morphotypes collected from Nubra valley revealed high genetic diversity. RAPD markers show five major groups in the natural population. None of the morphotypes shares more than 25 per cent similarity indicating a high level of genetic diversity between the morphotypes. Recently, seventeen natural population of *H. rhamnoides* (*N*=187 individuals) from major distribution sites of Leh and Nubra valley were studied for genetic diversity using 20 quantitative morphological characters and molecular markers (AFLP)[8]. A significant correlation was found between morphological and genetic data. Topography of the region mainly affects the genetic structure of SBT population in the region. The Ladakh mountain range (6500 m amsl) acts as natural barrier to gene flow and splits the genetic pool into two clusters- Leh and Nubra. Populations from Leh valley showed higher genetic diversity as compared to that of Nubra valley. Altitude plays no significant role on genetic diversity.

Raina[9], *et al.* compared the diversity and relationships of *H. rhamnoides*, *H. salicifolia* and *H. tibetana* from Indian Himalayas using AFLP and SAMPL. The study revealed that *H. rhamnoides* ssp. *turkestanica* from Ladakh and Lahaul-Spiti are the most diverse, and showed extreme divergence from those of Uttrakhand. The average genetic distance between *H. rhamnoides* from Ladakh and that of Uttrakhand genotypes is much greater than that obtained among the three species.

2.4 Cytology and Reproductive Biology

Diploid chromosome number of *H. rhamnoides* is 2n=24. The chromosome length ranges between 57.7 and 65.2 µm[10].

Figure 1. Morphological diversity of *H. rhamnoides* in Ladakh.

SBT is predominantly a dioecious species. However, sexual polymorphism is prevalent in some populations where plants are either hermaphrodite or monoecious or bear hermaphrodite flowers in addition to male and female on the same plant[10]. The flowers lack nectar and fragrance. Wind is the exclusive mode of pollination[11]. Flowering occur between mid April and first week of May when the plants are completely leafless. The male flowers are 3.4±0.1 mm in length and each is represented by four stamens enclosed in two perianth lobes. Each flower produces 81,714±1894 pollen which dispense in 2-3 days. The pollen grains lose their viability 72 h after anther dehiscence[11]. Female flower is represented by a gynoecium covered with two partly fused parianth lobes. Each flower produces a single ovule. During anthesis (0900-1200 h) the stigma emerges from the parianth lobes and remains most receptive at the time of anthesis[11]. Anthesis initiates during second to last week of April. Anthesis in male plants precedes the female plants by 24 h to 48 h[10], which ensure the availability of airborne pollen grains when the female flowers are receptive. Pollen travel up to 15 m of distance, therefore, it is recommended that the distance between female and male plant be maintained within 10 m - 15 m range for adequate pollination[11].

2.5 High Yielding Selection

SBT has not received much attention for breeding high yielding lines in India. One approach for selecting a superior genotype would be the screening of natural SBT population for desirable characters such as high berry yield, ease of berry harvest, lesser or no thorns etc. Extensive survey was conducted in Leh, Suru and Nubra valley in trans-Himalayan Ladakh and 186 genotypes were selected that yield more than 5 kg of berry per plant. The large collection has been narrowed down to six high yielding selections, which are being maintained at DIHAR Leh. Currently mass propagation of the selections is underway with an aim to promote cultivation of high yielding selections on large scale.

2.6 Propagation

There is a growing interest in the vegetative propagation of SBT primarily due to an increasing commercial demand for the plant. Successful plantation of the offspring of elite shrubs will require efficient propagation system in place to mass propagate superior genotypes. Vegetative propagation through hardwood cutting is the method of choice for propagation. Methods have been standardised for propagation of hardwood stem cuttings of the size of a pencil thickness (5-10 mm diameter)[12-13]. But this method has two major drawbacks for an efficient propagation. First, propagation by a pencil thick hardwood cutting results in fewer cuttings per plant; secondly the rooting success rate is low. Therefore, Dolkar[14], *et al.* developed an improved method using previous season's growth stem cutting (2.9±0.8 mm thickness) for propagation of SBT. The improved method resulted in much higher rooting percentage than those reported for conventional method using a pencil thick hardwood cutting. It emerged that 2.9 mm thickness SBT cutting can be rooted at reasonably high rates without exogenous auxin treatment. Additionally, seven times more number of cuttings can be taken from each shrub as compared to that of a pencil thick hardwood cutting. Therefore, the improved method facilitates establishment of a vegetative propagation wherein a larger number of cuttings per plant can be propagated with higher rooting success without the need for exogenous application of rooting hormone.

SBT has a profuse suckering habit. A single plant produces 13-65 suckers which can be separated from the mother plant[15]. The individual suckers have been used as a true to type planting material. Air layering has also been found useful in raising SBT nurseries. Micropropagation of SBT has been standardised in Murashige & Skoog media. For shoot proliferation 0.01 ppm IBA is found optimum while 2.0 ppm BA and 1.0 ppm NAA is optimum for rooting[15].

SBT can also be propagated by seed. However, the method is not preferred for propagation of plants for commercial cultivation. Freshly harvested seeds have a short physiological dormancy and thus do not germinate immediately after harvesting. SBT seeds can be kept satisfactorily up to six years without significant loss of viability at room temperature in Ladakh condition. However, seed aged 9 and 10 years shows significant reduction in germination percentage of 65.3% and 65.67%, respectively compared to 100% and 99% in one and two years old seeds, respectively[16]. In contrast, it has been reported elsewhere that dry seeds can be keep satisfactorily for only 1 to 2 years at room temperature. In another study, 60 per cent viability has been reported for seeds stored for 4 to 5 years. Higher seed germination of aged seeds in the study could be due to prevailing lower temperature and dry conditions of Ladakh. The phenomenon may have ecological importance since SBT is a pioneer plant species and it is selectively advantageous to maintain high germination rate stretched over a period of time to offset unfavorable conditions for germination in adverse conditions. The study suggested that short and medium term storage of seed could be achieved at ambient condition in cold arid region at lower cost without the limitation of space[16].

2.7 Antimicrobial Activity

SBT seed, pomace and leaf extracts possess antimicrobial property. Arora[17], *et al.* studied the extracts against six Gram-positive and 11 Gram-negative foodborne and food spoilage pathogens. The leaf extract showed significantly higher antibacterial activity and showed antimicrobial activity against 16 reference strains, followed by seeds (12) and pomace (7). *In vivo* antilisteric test against carrot infected with *L. monocytogenes* suggested that leaves extract from SBT has a scope for the possible use as biosanitizer in food industries.

2.8 Bioactive Content

SBT growing in Leh valley of trans-Himalaya is found to be with a high concentration of vitamins including vitamin C (275 mg/100g), vitamin A (432.4 IU/100g), vitamin E (3.54 mg/100g), Riboflavin (1.45 mg/100g), Niacin (68.4 mg/100g), Pantothenic acid (0.85 mcg/100g), vitamin B-6 (1.12 mg/100g), and vitamin B-2 (5.4 mcg/100g). It is also found to be having high amount of minerals including potassium (647.2 mg/l), calcium (176.6 mg/l), iron (30.9 mg/l), magnesium (22.5 mg/l), phosphorous (84.2 mg/l), sodium (414.2 mg/l), zinc (1.4 mg/l), copper (0.7 mg/l), manganese (1.06 mg/l) and selenium (0.53 mg/l)[18].

Korekar[19], *et al.* studied the effects of various solvent extraction (water, methanol, acidic 50 per cent methanol, 70 per cent acetone, acidic 50 per cent methanol followed by 70 per cent acetone) on the total phenolic content (TPC) and antioxidant capacity of fruit pulp, seeds, leaves and stem bark of SBT. It was found that SBT extracts possess high phenolic content in terms of GAE/100g DW (1666-13769). The mean TPC is highest in seeds (11148) followed by stem bark (10469), leaves (6330) and pulp (3579 mg GAE/100g DW). In general, the 70 per cent acetone and acidic 50 per cent methanol followed by 70 per cent acetone extract is reported to contain significantly higher TPC than those obtained in other extracting solvents. Antioxidant capacity in terms of inhibitory concentration (IC_{50}) value of pulp (3.39 mg ml^{-1}) is up to 7.8 times higher than that of stem bark (0.43 mg ml^{-1}) and up to 2.4 times higher than that of seeds (1.4 mg ml^{-1}). Further, antioxidant capacity by ferric reducing antioxidant potential (FRAP) assay shows that the stem bark possess maximum antioxidant capacity (16.83) followed by seeds (15.26), leaves (12.73) and pulp (12.61 mM $FeSO_4$). Significant correlation is found between TPC and antioxidant capacity by DPPH and FRAP assays.

A total of 187 plants representing 17 natural populations of SBT from trans-Himalaya were studied to find out the variability and genotypic effect on TPC, total antioxidant capacity (TAC), ascorbic acid and carotenoids content in fruit pulp. The fruits are found to be rich in TPC ranging from 964 to 10704 mg gallic acid equivalent /100 g. The free radical-scavenging activity in terms of IC_{50} ranges from 0.7 to 9.1 mg/ml and FRAP from 180 to 1355 $FeSO_4.7H_2O$ µg/ml. The ascorbic acid and carotenoids content ranges from 56 to 3909 mg/100g and 0.1-14.4 mg/100 g, respectively. Variations in bioactive content among the examined fruit across 17 populations underline the vital role played by genetic background and the geographical location for determining the health promoting compounds. Significant correlation has been observed between TPC, IC_{50}, FRAP, carotenoids, ascorbic acid, fruit lightness (L*) and plant height. Plant height and fruit colour has significant positive correlation with TPC and TAC suggesting interrelationship between morphological and biochemical traits[20].

Ranjith[21], *et al.* reported fatty acid composition, carotenoids and tocols of pulp oil. The dominating fatty acid are palmitoleic (46 per cent - 49 per cent), palmitic, oleic, linoleic and lenolenic acids. The proportion of the rare 16:1 fatty acid in pulp oil is significantly higher in *H. rhamnoides* as compared to *H. salicifolia* and *H. tibetana*. The carotenoids in pulp oil of *H. rhamnoides* ranges from 2350-2650 mg/kg, which is significantly higher than that of *H. salicifolia* (692-840 mg/kg). The amount of tocols in pulp oil varied from 1301 to 1394 mg/kg in *H. rhamnoides*, which is significantly higher than that of *H. salicifolia* (666-902 mg/kg).

The influence of plant genetic background on TPC, TAC and total flavonoid content (TFC) in SBT seed was demonstrated by Dolkar[22], *et al.* in nine natural population of SBT (122 plants). Significant variation is found within and between the natural populations. TPC ranges from 32 to 208 mg gallic acid equivalent/g DW, while flavonoid content is 8 to 38 mg quercetin equivalent/g of DW. The free radical-scavenging activity in terms of inhibitory concentration (IC_{50}) ranges from 0.3 to 9.2 mg/ml and ferric reducing antioxidant potential (FRAP) from 11 to 97 mmol Fe (II)/g. The study demonstrates the role of both genetic background and environmental factors for determining the health promoting compounds in SBT seed.

Dolkar[23], *et al.* reported gender differences and seasonal variation in TPC and TAC in SBT leaf. Male exhibits significantly higher TPC (100.8±23.9 mg GAE/g DW) than female (95.0±23.8 mg GAE/g DW). Similarly, ferric reducing activity is reported significantly higher in male (6.5±1.1 Fe^{2+} mmol/g DW) than female (6.1±1.2 Fe^{2+} mmol/g DW). TPC in male leaves was seen on an increasing trend from July to October followed by a significant decrease in November. However, in female leaves the TPC is observed to be on increasing trend upto August and then on a steady declining trend. Similar trend is observed in TAC in both the sexes except that female also shows increasing TAC trend from July to October. The study concludes that male exhibit higher TPC and TAC than female, and that October is the best time for harvesting SBT leaves. Gupta and Kaul[24] reported presence of phenol, flavonoids, tannins, sterols, saponins, ascorbic acid, alkaloids, cardiac glycosides, terpenoids and flavonols in both male and female SBT leaves. A higher concentration of N, P, K and Cu was found in leaf of the plants growing in higher altitude than those from lower altitude[25].

2.9 Tricotyledony in *H. rhamnoides*

The phenomenon of typically dicotyledonous plants producing three cotyledons has been referred to as tricotyledony or tricotyly. Normally the production of an abnormal number of cotyledons has been referred to as pleiocotyly. Tricotyledonous seedlings occur sporadically in nurseries of dicotyledonous plant species in over 15 families of plants. However, Korekar[26], *et al.* reported the phenomenon for the first time in SBT. The observed tricotyledon frequencies among the 2798 germinated seedlings from 30 plants ranges from 0 per cent to 6.4 per cent, with an average of 0.64 per cent. A rare single tetracotyledon seedling was also observed. None of the cotyledonary leaves show any sign of external distortion or splitting and were arranged symmetrically in a whorl. Seedlings bearing three cotyledons also bear three true leaves at each internode of the first few internodes.

2.10 Sex-linked SCAR Marker

SBT is a wind pollinated dioecious crop. For commercial berry production, the female plant is critical whereas 10 per cent male plants are needed in the field to produce fertile pollen. Unfortunately gender of SBT seedlings cannot be determined until flowering, which usually takes place 3-4 years after planting in the field. Early sex determination of SBT has commercial application. Both money and time can be saved if undesired male/ female plants can be eliminated at an early stage of research trials and commercial plantation. In this context, Korekar[27], *et al.* reported two robust sex-linked sequence-characterised amplified region (SCAR) markers in SBT. The markers, namely *HrX1* and *HrX2*, were validated in larger sets of female (120) and male (100) genotyped and are proved to be perfectly linked to female sex in *H. rhamnoides*. The markers were later checked for cross-species amplification in *H. salicifolia* and *H. tibetana*. It is found that *HrX1* is specific to *H. rhamnoides* while *HrX2* is universal and it works across three species i.e. *H. rhamnoides*, *H. salicifolia* and *H. tibetana*[28]. Das[29], *et al*. recently reported a male-specific SCAR marker, which was validated on 47 male and 45 female *H. rhamnoides* from Ladakh.

2.11 Transcriptome Analysis

SBT is a hardy plant, and the shrub is extensively studied to answer the key questions pertaining to abiotic stress management. Ghangal[30], *et al.* reported generation of transcriptome data that provides information about genes involved in abiotic stress. Out of 1665 unigenes, 43 unigenes are found to be responsive to biotic and abiotic stresses. Ghangal[31], *et al.* reported the use of next generation massive parallel sequencing technology and *de novo* assembly to gain a comprehensive view of the SBT transcriptome. A total of 86,253,847 high quality short reads was assembled using six assembly tools. The assembled transcripts were screened for the presence of transcription factors and simple sequence repeats. Chaudhary and Sharma[32] employed DeepSAGE, a tag based approach to identify differentially expressed genes under cold and freeze stress in SBT. The study provides a comprehensive view of global gene expression profile of SBT under cold and freeze stresses.

2.12 Processing of SBT Berry

There is a dearth of reports describing the processing of SBT berry. Freshly harvested berries are highly perishable and long distance transportation requires maintenance of cold chain. Therefore, primary processing of fresh SBT berry is being done at the site of berry collection and various other components such as the pulp, seed and hull are segregated and sold separately for further value addition outside the region. Due to remoteness the use of sophisticated equipments is challenging and economically unviable.

SBT berry is manually harvested in the month of September using 'beat the bush' method. A plastic sheet is placed on the ground under the canopy. Tip of a branch is held with one hand and beaten with a stick with the other. The berries that fall on the plastic sheet are collected in a bucket and transported to the processing unit in 10 kg capacity food grade plastic tray. It is then cleaned by washing in potable water while removing damaged berries and other foreign bodies which might have been fallen into it during harvesting. Juice extraction from berry is done in a pulper. When cleaned berries are fed into the pulper, pulp (73.8±3.3 per cent) and cake (24.1±3.0 per cent) containing unbroken seeds are obtained. The residual cake containing unbroken seed is sun dried and the seed is separated by rubbing the dried residue with hands to get clean seed and the hull. Drying of whole berry is also carried out in view of high demand for dried whole berry in recent years. On an average, 33.3±3.1 kg of dried whole berry is obtained when 100 kg of cleaned berry is sun dried. A flow chart for processing of fresh SBT berry is shown at Fig. 2[33].

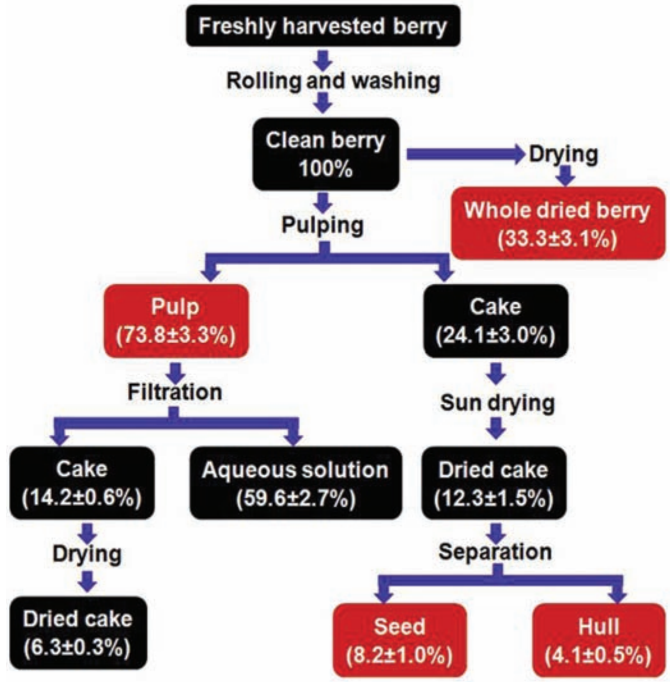

Figure 2. Primary processing of fresh SBT berry. Values in bracket show the percentage (w/w) of various components obtained from processing of 100 kg of clean berry. Products in red box are generally sold from Ladakh for further value addition outside the region (adapted from Stobdan[33], *et al*.).

2.13 Product Development

High altitude increases the production of free radical that damages the cells. Antioxidants can reduce the amount of damage to human body caused by altitude exposure. DIHAR has developed several antioxidant rich products from fruit and leaf of SBT to combat high altitude related oxidative stress. Patented SBT-based commercialised products include Seabuckthorn beverage (patent no. 231773), Seapricot beverage (patent no. 635/DEL/2009), Herbal tea (patent no. 242959), Oil capsule (patent no. 1430/DEL/2011), Seabuckthorn jam (patent no. 635/DEL/2009), Antioxidant herbal supplement (patent no. 635/DEL/2009), and Adaptogenic appetizer (987/DEL/2012). Commercialisation of the products since the year 2001 has resulted in huge demand for SBT. However, limited natural resource of SBT remains a major limiting factor in product commercialisation.

2.14 Income Generation

SBT berry collection has become an important income generating activity in the region since year 2001. The mean annual berry harvest from 2004 to 2015 was 213.4±83.9 MT, which is less than 5 per cent of the total available SBT resource in the region. Berry harvesting is done for a short period of 20-30 days in September. In year 2013, 0.8 per cent of the total population of Leh district have directly benefitted from berry collection. Average collection per collector was 254.8 kg resulting in a net income of Rs 8,154 per person in 5-10 days. However, the average income generation of individuals who devoted an average of 12.6 days in the season was Rs 18,375 per head. Majority of the berry collectors are from the needy section of the society and women constitute 67.4 per cent of the work force[34]. Currently, the demand for SBT exceeds the supply capacity of the region. With the increasing trend in demand for SBT, the berry harvest from available resource is expected to increase to 1509 MT in 2030 from current 361.3 MT[35]. Therefore, SBT has the potential to become an important means for sustainable livelihood in Ladakh.

Plantation of SBT on barren land by Forest Department and local community for greening and income generation is slowly gaining momentum. The vast barren land in the region can be brought under SBT plantation either by planting along existing water sources or through lifting of water for irrigation from the river. According to an estimate of the Forest Department, 2500 ha of barren land can be brought under SBT plantation without much investment in Leh district. If cultivation is done on 2500 ha in a planned manner the projected net income from SBT alone is estimated to be Rs 491 crore (USD 72 million) in the year 2030[35]. Income generation will increase many-fold if value added products are also manufactured in the region.

3. CONCLUSION

SBT is an ecologically and economically important plant species for cold arid region of Ladakh. Several products have been developed and commercialised, which immensely benefitted the modern society from the lesser-known shrub. Immediate technological intervention is required on method of berry collection, drying and systematic plantation. SBT plantation would be highly profitable and will also become a means for sustainable development of Ladakh region. Potential of the lesser-known shrub has been recognised by several R&D organisations. In recent years a number of research institutes in India have started working on SBT. Collaborative research is required on mission mode to further strengthen R&D on SBT. The plant serves as a storehouse for researchers in the field of biotechnology, neutraceutical, pharmaceutical, cosmetic and environmental sciences.

Conflict of Interest: None

REFERENCES

1. Stobdan, T.; Korekar, G.; Dwivedi, S.K.; Yadav, A.; Targais, K. & Singh, S.B. Research on seabuckthorn *(Hippophae rhamnoides* L.) in India. *In* advances in agriculture, environment & health, edited by S.B. Singh, O.P. Chaurasia, A. Yadav, A.M. Rimando & T.H. Terrill. SSPH Delhi, India, 2009, pp. 83-97.
2. Stobdan, T.; Angchuk, D. & Singh, S.B. Seabuckthorn: An emerging storehouse for researchers in India. *Curr. Sci.*, 2008, **94**, 1236-7.
3. Singh, R. Ethnobotany, anatomy and bio-chemical studies on seabuckthorn (*Hippophae rhamnoides* L.) in Nubra valley of Ladakh. Maharshi Dayanand University, Rohtak, India, 2004. (Ph.D Thesis)
4. Tamchos, S. & Kaul, V. Distributional status of *Hippophae tibetana*: a new report from Changthang valley of Ladakh. *Natl. Acad. Sci. Lett*., 2016, **39**, 59-62. doi:10.1007/s40009-015-0396-y
5. Chauhan, K.; Khare, S. & Jain, S.C. Seabuckthorn (*Hippophae*) mapping in Ladakh region of Jammu and Kashmir. *In* Symposium of International Society for Photogrammetry and Remote Sensing. Vol XXXVI, Part 4, 2006.
6. 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. *Natl. Acad. Sci. Lett*., 2013, **36**, 609-13. doi:10.1007/s40009-013-0177-4
7. Singh, R.; Mishra, S.N.; Dwivedi, S.K. & Ahmed, Z. Genetic variation in seabuckthorn (*Hippophae rhamnoides* L.) population of cold arid Ladakh (India) using RAPD markers. *Curr. Sci.*, 2006, **91**(10), 1321-2.
8. Korekar, G. Genetic diversity of Seabuckthorn (*Hippophae rhamnoides* L.) in trans-Himalayan Ladakh region, India. Jaypee University of Information Technology, Solan, India, 2013. (Ph.D Thesis)
9. Raina, S.N.; Jain, S.; Sehgal, D.; Kumar, A.; Dar, T.H.; Bhat, V.; Pandey, V.; Vaishnavi, S.; Bhargav, A.; Singh, V.; Rani, V.; Tandon, R.; Tewari, M. & Mahmoudi. A. Diversity and relationships of multipurpose seabuckthorn (*Hippophae* L.) germplasm from the Indian Himalayas as assessed by AFLP and SAMPL markers. *Genet. Resour. Crop Evol*., 2012, **59**, 1033-53. doi: 10.1007/s10722-011-9742-1
10. Ali, A. Assessment of morphological and cytological variability in seabuckthorn (*Hippophae rhamnoides* L.) from some areas of Ladakh. University of Jammu, Jammu, India, 2013. (Ph.D Thesis)
11. Mangla, Y. & Tandon, R. Pollination ecology of Himalayan sea buckthorn, *Hippophae rhamnoides* L. (Elaeagnaceae). *Curr. Sci.*, 2014, **106**(12), 1731-5.
12. Dwivedi, S.K.; Paljor, E.; Attrey, D.P. & Singh, B. Propagation of common seabuckthorn (*Hippophae rhamnoides*) through hard wood cutting in Ladakh. *Indian J. Agr. Sci.*, 2002, **72**, 228-9.
13. 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.
14. 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
15. Dwivedi, S.K.; Singh, R. & Ahmed, Z. *The Seabuckthorn*. Field research laboratory (DRDO), Leh-Ladakh, India, 2006, 57 p.
16. Korekar, G.; Dwivedi, S.K.; Singh, H.; Srivastava, R.B. & Stobdan, T. Germination of *Hippophae rhamnoides* L. seed after 10 years of storage at ambient condition in cold arid trans-Himalayan Ladakh region. *Curr. Sci.*, 2013, **104**, 110-14.
17. Arora, R.; Mundra, S.; Yadav, A.; Srivastava, R.B. & Stobdan, T. Antimicrobial activity of seed, pomace and

leaf extracts of seabuckthorn (*Hippophae rhamnoides* L.) against foodborne and food spoilage pathogens. *Afr. J. Biotechnol.*, 2012, **11**(45), 10424-10430. doi:10.5897/ajb11.4150

18. Stobdan, T.; Chaurasia, O.P.; Korekar, G.; Mundra, S.; Ali, Z.; Yadav, A. & Singh, S.B. Attributes of seabuckthorn (*Hippophae rhamnoides* L.) to meet nutritional requirements in high altitude. *Def. Sci. J.*, 2010, **60**, 226-30. doi: 10.14429/dsj.60.344
19. Korekar, G.; Stobdan, T.; Chaurasia, O.P. & Singh, S.B. Phenolic content and antioxidant capacity of various solvent extracts from seabuckthorn (*Hippophae rhamnoides* L.) fruit pulp, seeds, leaves and stem bark. *Acta Aliment*., 2011, **40**(4), 449-58. doi: 10.1556/AAlim.40.2011.4.4
20. Korekar, G.; Dolkar, P.; Singh, H.; Srivastava, R.B. & Stobdan, T. Variability and the genotypic effect on antioxidant activity, total phenolics, carotenoids and ascorbic acid content in seventeen natural population of seabuckthorn (*Hippophae rhamnoides* L.) from trans-Himalaya. *LWT-Food Sci. Technol.*, 2014, **55**, 157-62. doi: 10.1016/j.lwt.2013.09.006
21. Ranjith, A.; Kumar, K.S.; Venugopalan, V.V.; Arumughan, C.; Sawhney, R.C. & Singh. V. Fatty acids, tocols, and carotenoids in pulp oil of three sea buckthorn species (*Hippophae rhamnoides*, *H. salicifolia*, and *H. tibetana*) grown in Indian Himalayas. *JAOCS*, 2006, **83**, 359-64. doi: 10.1007/s11746-006-1213-z
22. Dolkar, P.; Dolkar, D.; Angmo, S.; Kumar, B. & Stobdan, T. Variability in phenolics, flavonoids and antioxidants in seabuckthorn (*Hippophae rhamnoides* L.) seed from nine trans-Himalayan natural population. *J. Berry Res*., 2017. doi: 10.3233/JBR-170149
23. Dolkar, P.; Dolkar, D.; Angmo, S.; Kant, A.; Kumar, B. & Stobdan, T. Sexual differences and seasonal variations in total phenolics and antioxidant properties in (*Hippophae rhamnoides* L.) *J. Berry Res*., 2017. doi: 10.3233/JBR-170147
24. Gupta, D. & Kaul, V. Qualitative analysis of bioactive compounds in leaves of (*Hippophae rhamnoides* L.) *Natl. Acad. Sci. Lett*., 2013, **36**, 477-81. doi: 10.1007/s40009-013-0160-0
25. Sharma, V.K.; Dwivedi, S.K.; Awasthi, O.P. & Verma, M.K. Variation in nutrient composition of seabuckthorn (*Hippophae rhamnoides* L.) leaves collected from different locations of Ladakh. *Indian J. Hort*., 2014, **71**(3), 421-3.
26. Korekar, G.; Singh, H.; Srivastava, R.B. & Stobdan, T. Tricotyledony in (*Hippophae rhamnoides* L.) (Elaeagnaceae). *Curr. Sci.*, 2012, **25**(2), 159-60.
27. Korekar, G.; Sharma, R.K.; Kumar, R.; Meenu; Bisht, N.C.; Srivastava, R.B.; Ahuja, P.S. & Stobdan, T. Identification and validation of sex-linked SCAR markers in dioecious (*Hippophae rhamnoides* L.) (Elaeagnaceae). *Biotechnol. Lett.*, 2012, **34**(5), 973-8 . doi: 10.1007/s10529-012-0852-4
28. Chawla, A.; Kant, A.; Stobdan, T.; Srivastava, R.B. & Chauhan, R.S. Cross-species application of sex linked markers in *H. salicifolia* and *H. tibetana*. *Sci. Hortic.*, 2014, **170**, 281-3. doi: 10.1016/j.scienta.2014.03.013
29. Das, K.; Ganie, S.H.; Mangla, Y.; Dar, T-U-H.; Chaudhary, M.; Thakur, R.K.; Tandon, R.; Raina, S.N. & Goel, S. ISSR markers for gender identification and genetic diagnosis of *Hippophae rhamnoides* ssp. *turkestanica* growing at high altitudes in Ladakh region (Jammu and Kashmir). *Protoplasma*, 2016, doi:10.1007/s00709-016-1013-8
30. Ghangal, R.; Raghuvanshi, S. & Sharma, P.C. Expressed sequence tag based identification and expression analysis of some cold inducible elements in seabuckthorn (*Hippophae rhamnoides* L.). *Plant Physiol. Biochem*., 2012, **51**, 123-8. doi: 10.1016/j.plaphy.2011.10.005
31. Ghangal, R.; Chaudhary, S.; Jain, M.; Purty, R.S. & Sharma, P.C. Optimization of De Novo short read assembly of seabuckthorn (*Hippophae rhamnoides* L.) transcriptome. *PLoS ONE*, 2013, **8**(8), e72516. doi:10.1371/journal.pone.0072516
32. Chaudhary, S. & Sharma, P.C. DeepSAGE based differential gene expression analysis under cold and freeze stress in seabuckthorn (*Hippophae rhamnoides* L.). *PLoS ONE*., 2015, **10**(3), e0121982. doi:10.1371/journal.pone.0121982
33. Stobdan, T.; Targais. K.; Dolkar, D.; Dolkar, P.; Angmo, S. & Kumar, B. Seabuckthorn in trans-Himalayan Ladakh: Primary processing and income generation. *In* Proceedings of 7th Conference of the International Seabuckthorn Association, New Delhi, India, 2015, pp 14-18.
34. Stobdan, T. Seabuckthorn plantation for sustainable development of trans-himalayan Ladakh. *In* Defence Technology Spectrum. Defence Research and Development Organisation, Ministry of Defence, India, 2016, pp 141-144
35. Bindal, M.; Stobdan, T. & Kumar, B. Seabuckthorn cultivation for sustainable development of trans-himalayan Ladakh: Projected income from 2016-2030. *In* National Seminar on Conservation and Sustainable Utilization of trans-Himalayan Medicinal Plants, Leh Ladakh, India, 2016.

CONTRIBUTORS

Dr Tsering Stobdan is Scientist 'E' and Head, Plant Science Division at DIHAR, Leh. He received his PhD in Molecular Biology & Biotechnology from Indian Agricultural Research Institute, New Delhi. He has 5 patents including one in USA, over 40 publications in reputed national and international journals, two monograph and 20 book chapters to his credit. Two technologies developed by him have been successfully transferred to Industries. .

Ms Phuntsog Dolkar is Senior Research Fellow at DIHAR, Leh. She is working on gender differences in Seabuckthorn in trans-Himalayan Ladakh.

Dr O.P. Chaurasia is Director, Defence Institute of High Altitude Research. He obtained his PhD (Botany) from Magadh University, Bodh Gaya, Bihar in 1992. He has extensively surveyed trans-Himalayan belts of Ladakh and Lahaul-Spiti and documented the fragile plant biodiversity and its ethnobotanical wealth. Two research fellows have been awarded PhD under his supervision.

Dr Bhuvnesh Kumar is Director, Defence Institute of Physiology and Allied Sciences, Delhi. He obtained his graduate degree in Veterinary Sciences (BVSc & AH), MVSc and PhD in Veterinary Medicine from G.B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand) in the year 1982, 1984 and 1999, respectively. He has been a member of G-FAST from Life Science cluster, Project Director of LIC Programme and Director, PM (LS). He has vast experience of working in mountainous regions covering western, central and north east Himalayas.