Frankia Nodulation Study of *Hippophae salicifolia* in Riverine and Non-riverine Areas of Lachen Valley in North Sikkim

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ABSTRACT

The study was carried out to compare various factors affecting the nodulation by actinomycetes *(Frankia)* in the roots of *Hippophae salicifolia* growing in riverine and non-riverine areas. The root nodules numbers, weights, soil moisture, plant age, plant height, plant canopy and sizes of the root nodules were determined in a 3-6 years old plant with an altitude ranging from 2540 to 3099 m. Ten trees each from both the areas were selected for the study. Healthy and maximum numbers of nodules were found at a depth range of 4-6 inches. ANOVA of number of root nodules and weight of root nodules found highly significant P0.00096, P-0.0066 respectively. Positive correlation was analyzed between number of nodules and the soil moisture content in both the areas. No relationship was found between the weight and the age of the nodules. Soil analysis (org C, K_2O, P_2O_5, S and Cl) found to be less in riverine areas when compared to non-riverine areas. Maximum number of nodulation was found in riverine area (580 nos.) as compared to non-riverine area (224 nos.). This may be due to deficiency of nutrient in host plant, thus larger nodules are formed to compensate their requirement of nutrients.

Keywords: *Hippophae salicifolia,* nodulation, riverine, non-riverine, Lachen and Sikkim.

INTRODUCTION

Seabuckthorn *(Hippophae salicifolia* D. Don) belongs to family *Eleagnaceae* (2n=24) is an actinorizal angiosperm, which colonizes in a new soil (Basistha *et al.,* 2009). The genus is divided into seven species and nine subspecies based on its morphological variations (Bartish *et al.*, 2002). It is spread almost the entire Eurasian continent from Central China, across the Himalayas, in a more or less continuous belt through the mountain valleys of central, southern and western Asia to the Black Sea. In India, the plant is generally found in the higher Himalayan regions of Himachal Pradesh, Jammu and Kashmir, North East region and Uttarakhand.

H. salicifolia is a thorny, deciduous, dioecious tree (Singh, 1998; Basistha *et al.*, 2009). It is tallest among all species of seabuckthorn. In addition to its high medicinal and nutraceutical properties, seabuckthorn is also valued for its high ecological roles in nature (Singh, 1998). *H. salicifolia* is an economically important plant that grows in the higher Himalayan region (Bartish *et al.,* 2002). The plant plays important role in preventing soil erosion and soil water loss in regulating microclimate, as well as in retaining stability in the region (Rogen, 1992). *Hippophae* L. has been shown to be superior to any other tree in afforestation, soil conservation, wasteland reclamation, (Wheeler *et al.,* 1986), especially on fragile slopes due to its extensive root system. It is also a multipurpose fast growing species, which is serves as a measure of biodiversity conservation, soil conservation, medicines, food, fodder and fuel wood. *H. salicifolia* is an important genus in the succession of vegetation. It grows in riverine (river side) and non-riverine (non riverside) areas in open natural habitat away from river and stream banks (Basistha *et al.,* 2009).

This actinorhizal species has the capacity of holding soil in fragile slopes, needs no good arable land for its cultivation and has symbiotic relationships with nitrogen fixing actinomycetes known as

Frankia in its root nodule (Akkermans *et al.,* 1983; Dobrista and Novik, 1992; Rongsen, 1992; Basistha, 2009). Akkermans and Dijk, 1975 reported that the highest nodulation was found in 1 to 2 year old plants, older plants are poorly nodulated, though they were frequently connected by stolons with nodulated young shoots. The nitrogen fixation effect of seabuckthorn root nodules varies with seasons, precipitation, age of plant and environmental factors (Basistha *et al.*, 2009). Oremus, 1979 reported that there was a sharp decrease in the number of nodules in the age classes of four years and older, which indicated that many of them died after three years. Of the total number of living nodule material 89% were younger than four years. Changes occur even in a day, between morning and evening (Zhou *et al.,* 1999). Oremus, 1979 also described variation in the number of nodules in different plots might be partly due to the difference in chemical composition of the soil. However, the results showed that for these factors the differences in the samples were small and they were very unlikely to be responsible for the strong variation in nodule number.

Thus in the present study, Lachen valley was taken as the study area as it has a very interesting admixture of land cover and land use practices. The canopy varies from as low as 10% in shrub areas to almost 80% in dense subtropical forest. The reverine area is mostly dominated by seabuckthorn forest whereas the non-riverine side has mixed forest. As the nodulation of *H. salicifolia* appeared to be highly variable, and also the studies shows that the nodules in homogenous scrub differ widely from place to place, the present study was undertaken to determine its nodulation frequency in the Reverine and Non-Reverine areas and also to check the effect of various factors like plant height, canopy, tillering and soil pH and chemical composition on nodulation.

MATERIAL AND METHODS

Study Area

Seabuckthorn study area consisted of approximately 300 ha, located in Lachen Valley (North District of Sikkim), which lied between 27^{0}47'32.1" and 27^{0}43'57.2" North latitude and 88^{0}33'07" and 88^{0}33'13" East logititude. The study of nodulation was carried out in the trees aged between 3-6 years in riverine (along side of the river) and non-riverine (away from river side) areas; ten trees each from both the areas were selected for study. GPS locations of all the boundary of seabuckthorn patches were recorded. These locations were sometimes, analyzed to find the distribution area of seabuckthorn using geocoded Indian Remote Sensing 1D LISS III, False Colour Composite (FCC), January 2012 data of 1: 50000 scales.

Mapping of nodule position

The area of 1.5 m around the tree and 6-8 inch below (rhizospheric region) the soil surface was dug carefully, the root system was extracted and position of nodules was mapped.

Data collection

Following a stratified random procedure, ten seabuckthorn trees each from nine diameter classes were selected. The dead dying and malformed trees were avoided. Measurements were taken for plant height using Laser range finder/ hypsometer model-400XL/XT/LH (Range-up to 400+yards passive 999 yards reflective), tillering (number of branches from the base) was also recorded through optical counting, girth, plant canopy using standard measuring tape, topography, site cover, vegetation type management, age of plant by Dendrochronology (Oremus, 1979) and were recorded. Simultaneously soil pH (pH meter-Takemura Electric Works LTD -Model No. -DM-15), air temperature (Multi thermometer, CE), aerial humidity (Model LR6 Mignon AA, England) was measured.

Measurement of nodules

Plants that were 3 to 6 years old and growing in an altitude range of 2540 m to 3099 were selected. In terms of their internodular distance, depth of nodules, nodule location and the growth of nodule were determined. Randomly 30% of the nodules were detached from the main root and their total weight was determined using top pan balance (Docbel, Brawn make) and their size was measured using a measuring tape.

Tagging of the sample

The tree was then tagged by numbering it using aluminum plant tag and wire for further studies of the same trees in different seasons. All the data were tabulated (Table 1-5, Figure 1) and evaluated.

RESULTS AND DISCUSSIONS

Results showed that maximum nodulation in *H. salicifolia* were observed in the tap root with few in lateral roots. Healthy and maximum numbers of nodules were found at a depth range of 4-6 inches. The nodules found in the lesser depth region were matured and dried as compared to those found in deeper region. It was observed that the nodules found in sandy soil were larger in size (clumped) and less in number as compared to the nodules found in humus soil, which were scattered and more in number but smaller in size. Calculations showed that the total number of nodules varied from 4 to 107 in different trees (Table 3 and 4). ANOVA of riverine and non-riverine areas of number of root nodules and weight of root nodules found highly significant P0.00096, P-0.0066 respectively (Table 1).

Table 1. Significance of root nodules and weight in Riverine and Non-riverine Areas

Nodulation	Source of variation	DF	Sum of Sq.	Mean of Squares	*F* value
Number of nodules	Between Groups 1 Within Groups	6336.8 18	6336.8 7552.4	15.51363 408.4667	0.000963
Weight of nodules	Between Groups 1 Within Groups	88112.81 8	88112.81 168926.3	9.38889 9384.796	0.006683

Figure 1 Interprets that in riverine area sandy soil has more moisture content than the humus soil and in non-riverine humus soil has more moisture content. Hence, the graph shows that the soil with more moisture content gives maximum number of nodulation in *Hippophae salicifolia*.

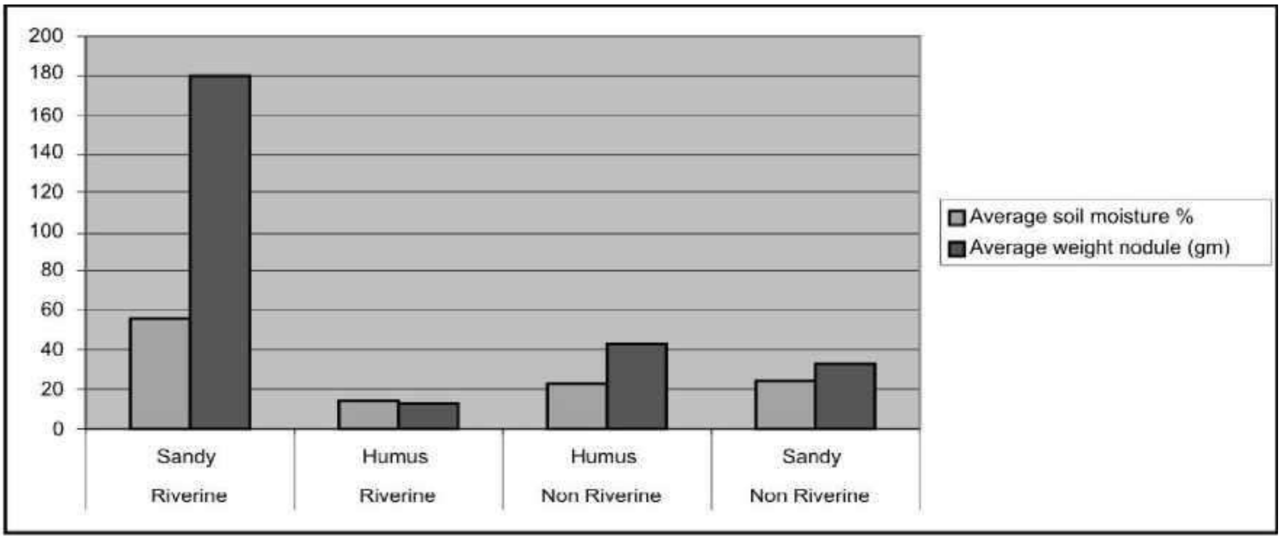

Figure 1. Effect of soil moisture and types of soil in nodulation in Riverine and Non-riverine area.

Positive correlation was analyzed between number of nodules and the soil moisture content in both the areas. No relationship was found between the mean nodule weight and the age of the nodules.

Earlier in a study it was showed that the chemical composition of soil is very unlikely to be responsible for the strong variation in nodule number in their respective study as the difference between the samples were small (Oremus, 1979). However in the present investigation the Soil analysis showed that (org C, K_2O, P_2O_5, S and Cl) was found to be less in riverine areas when compared to non-riverine areas.

The result of the Table 2 shows that in the riverine area the mean number of nodules in sandy soil was 56.33 with average weight of 179.16 gm and in the humus soil mean number of nodules was 52.75 with average weight of 13.33 gm. Similarly, in the non-riverine area the mean number of nodules in sandy soil was 18.71 and the mean weight 32.50 gm, and in humus soil it was 23.66 and the mean weight was 42.83 gm.

The data also shows that in the riverine area the moisture content of the sandy soil was 55.83% and of the humus soil it was 14.66%. In the non-riverine area the moisture content of sandy soil was 24.75% and of the humus soil was 28.01%. This may be due to deficiency of nutrient in the growing region of the host plant, thus larger nodules are formed to compensate their requirement of nutrients.

This data interprets that in the riverine area the number of nodules in sandy soil was more compared to the nodules in the humus soil but in non-riverine area the number of nodules in humus soil was more compared to sandy soil (Table 2). Close correlation was found between number of nodules and moisture content of the soil. From the data it can also be deduced that the moisture content and the type of soil plays an important role in nodulation. Hence the soil that had more moisture content gave high number of nodules compared to soil with low moisture content.

Table 2. Nodulation effect by soil moisture and types of soil

Area	Types of soil	Average soil moisture %	Mean No. of nodules	Average weight nodule (gm)
Riverine	Sandy	55.83	56.33	179.16
Riverine	Humus	14.66	52.75	13.33
Non Riverine	Humus	23.01	23.66	42.83
Non Riverine	Sandy	24.75	18.71	32.50

Quantitative study of nodulation was done earlier by some worker like, Stewart and Pearson, 1967 who found variation in nodulation from 98 and 370 nodules per square meter. In a study by Akkermans 1971, who investigated 32 m^2 one to two year old plant, nodule numbers varied from 9 to 200 per square meter. Same author found low level of nodulation in older plants (4-15 years), which varied from 0 to 25 nodules per square meter. However, these data were based on small study area. Oremus 1979, found nodulation varied from 30 to 281. All of these workers have done their work on the basis of different age group of plants. Stewart and Pearson, 1967 as well as Akkermans 1971, related their nodule number to the age of the vegetation by estimating the age of the shoot. Oremus, 1979 reported a close correlation between the age of the roots and the nodulation, young roots having the highest number. In this respect we based our studies on Quantative analysis of nodulation of different riverine and non riverine areas and the result of table 3 and 4 showed nodular quantity widely varied in riverine and non riverine areas. In riverine area nodulation varied from 22 to 107 and in non-riverine area it varied from 4 to 46.

Table 3 and 4 when compared, showed that the larger quantity no of nodules were found in the reverine area as compared to the non-riverine area. The data showed that there seemed to be a correlation in height of trees, canopy and soil pH for the nodulation. A negative correlation was observed between the tillering of the plant and nodulation. The sum of total nodules found at the riverine area was 580 and their weight was 1674.50 gm and in non-riverine area number of nodules was 224 and their weight was 347 gm.

Table 3. Nodulation data of Riverine area

Height (feet)	Canopy (feet)	Tillering	pH of soil	No. of Nodules	Weight of nodules	Inter nodular distance
15	10	1	6.5	61	350	6.7
15	9	1	6.2	29	150	8.2
11	6	1	6.2	22	100	3.9
13	10	1	6.8	41	45	4.5
22	5	1	6.8	107	140	4.5
20	20	2	6.5	76	9.5	3.2
35	20	1	6.8	45	400	6.5
15	10	2	6.8	66	110	13.2
15	12	1	6.6	78	300	4.1
11	5	1	6.8	55	70	2.3

Table 4. Nodulation data of Non-Riverine area

Height (feet)	Canopy (feet)	Tillering	pH of soil	No. of Nodules	Weight of nodules	Inter nodular distance
9	4	1	6.8	24	50	14.7
5	3	2	6.5	16	30	5.6
15	9	1	6.8	18	40	5.4
15	6	3	6.9	12	3	3.2
28	15.3	1	6.8	4	7	2.9
14	14.2	1	6.9	12	15	2.8
15	12	5	6.8	46	40	11.3
20	15	1	6.7	36	42	5.6
12	7	6	6.4	36	90	6.0
12	7	1	6.6	20	30	3.6

Earlier in 1979, Oremus in his studies described that the variation in the number of nodules in different plots might be partly due to the difference in chemical composition of the soil. Thus, in the present study the variations described above between the riverine and non-riverine area with respect to the number and the weight of the nodules can as well be due to difference in the chemical composition of the soil, therefore from each plot the soil was tested to determine its chemical composition. The result showed (Table No. 5 and 6) that the amount of organic carbon, average K_2O, average P_2O_5, average sulfur and chloride mg/gm were found to be less in riverine areas as compared to the non-riverine areas, as these chemicals are leached out in the riverine area due to a continuous flow of water.

Table 5. Nutrient analysis of Riverine Soil

EC m.mho/cm	Organic carbon	Avg. K_2O ppm	Avg. P_2O_5 ppm	Avg. Sulfur Ppm	Chloride mg/gm	Clay	Slit	Sand
0.11	0.57	38.43	21.76	11.78	0.002	34.00	4.00	85.00
0.14	0.78	46.97	21.68	22.59	0.005	28.00	3.00	88.87
0.14	0.97	42.70	15.23	23.57	0.004	30.00	5.00	90.90
0.16	0.49	64.05	19.56	25.35	0.005	20.00	2.00	93.98
0.06	0.64	67.10	26.88	25.53	0.005	27.00	2.00	75.00
0.23	0.86	71.98	26.87	24.12	0.004	26.00	4.00	78.09
0.23	0.86	76.25	28.29	21.60	0.005	22.00	3.00	88.98
0.19	0.95	80.53	26.66	24.55	0.004	35.00	2.00	86.75
0.23	0.41	78.08	26.67	26.51	0.005	26.00	2.00	83.98
0.23	0.35	78.08	26.11	26.51	0.005	34.00	2.00	96.98

Table 6. Nutrient analysis of Non-Riverine Soil

E.C m.mho/cm	Organic carbon	Avg. K_2O ppm	Avg. P_2O_5 ppm	Avg. Sulfur ppm	Chloride mg/gm	Clay	Slit	Sand
0.26	2.19	84.79	29.38	27.50	0.007	34.00	2.00	65.78
0.26	2.08	109.19	43.52	29.46	0.006	28.00	4.00	71.87
0.24	1.03	104.92	65.29	33.38	0.008	30.00	6.00	67.09
0.28	1.08	109.80	27.21	39.28	0.007	30.00	5.00	66.02
0.31	3.24	84.79	26.17	41.24	0.008	30.00	2.00	64.09
0.34	1.24	93.94	76.17	35.35	0.006	26.00	4.00	62.78
0.29	3.56	87.23	29.66	27.50	0.006	31.00	2.00	58.72
0.62	2.70	146.40	36.99	33.38	0.007	34.00	3.00	54.56
0.35	0.99	91.53	38.09	26.51	0.009	31.00	4.00	43.09
0.24	0.97	81.13	30.46	35.36	0.008	33.00	5.00	34.89

CONCLUSION

It can be concluded that the soil of the riverine area which is sandy may be deficient in nutrient content as the nutrient present in the soil may have been leached out by the continuous flowing river water, this may be the cause for less nutrient present in the soil of these area. Hence, it might be due to these reasons that the plants produce large number of nodules to compensate their nutrient requirement from root nodules for their growth. Also the moisture content in the soil might have enhanced the growth of the nodules to fix more N_2 to be supplied to the host plant.

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