Study of Seabuckthorn Root Nodule Nitrogen Fixation

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Abstract: The effects of season, direction of slope, age, and sex of plants on the activity of the nitrogen-fixing enzyme in seabuckthorn root nodules were studied. The results show that season is the main factor determining the activity of the nitrogen-fixing enzyme. The effectiveness of nitrogen fixation of the seabuckthorn root nodule on shady slopes is evidently related to plant age. On sunny slopes, the relationship is not evident. Age and sex of plant have no significant effect on nitrogen fixation. The average amount of nitrogen fixed in seabuckthorn forest is 30 to 60kg per hectare per year.
Key Words: Seabuckthorn, root nodule, nitrogen fixation

Introduction

Seabuckthorn is widely distributed in China from the Qinghai-Tibet Plateau in the southwest to the Daxingan Mountains in the northeast (Lian et al. 1992). About 1/3 of the total national seabuckthorn resources are located in Shanxi province (Seabuckthorn Office of the Yellow River Conservancy Commission 1998). Seabuckthorn has been shown to be superior to any other tree in afforestation, soil conservation, and wasteland reclamation (Zhao et al. 1990). The root nodule containing *Frankia* sp. is responsible for nitrogen fixation and soil fertilization. The nitrogen fixation effect of seabuckthorn root nodules varies with season, precipitation, age of plant, and environmental factors. Changes occur even in one day, between morning and evening (Zhou et al. 1985). There is very limited scientific information and therefore a need to research the activity of the nitrogen fixation enzyme of the seabuckthorn root nodule.

1. Materials and Methods

The forest is located in Wangjiashan, Gedun City, Fangshan County, Shanxi Province. It is a natural seabuckthorn forest with average growth, a canopy density of 0.5, and 3000 plants or clumps per hectare. The location is at 1200 meters above sea level, with 18 degrees of slope, loamy soil, and a pH of 7.8. The seabuckthorn species is *Hippophae rhamnoides* ssp*. sinensis.*

Starting in June, when seabuckthorn has exuberant foliage, through November, when leaves begin to fall down, root nodules of different age and sex on southern and northern slopes were tested for nitrogen fixation enzyme.

Two gram of one-year old fresh root nodule samples were placed in bottles containing streptomycin and sealed. Two ml of air from the sealed bottle were injected into 2 ml of acetylene for reaction at 35°C for 2 hours. The reaction was stopped by pouring the mixture into saturated salt water. To determine the activity of the nitrogen fixation enzyme (ANFE), the quantity of ethylene restored from acetylene was measured by Daojin GC-5A gas chromatograph (Zhou et al. 1990).

2 Results and Discussion

2.1 Effect of season on root nodule nitrogen fixation

The results in Table 1 show that ANFE was at a peak period when the seabuckthorn leaves were fully developed in June, and continued to last for 4 months under normal conditions. In October, when temperature and sunshine decreased and the leaves begin to age, root nodule nitrogen fixation declined. In November, when leaves turned yellow, no enzyme activity could be measured. Statistical analysis showed the season to be a significant factor for enzyme activity.

Table 1. The effect of season on root nodule nitrogen fixation

Month	No. of samples	Activity of nitrogen fixation enzyme (n molC_2H_4/(g x min))	F
6	29	179.40	$F_{0.01}$=13.46
7	39	126.60	
8	36	162.35	
9	36	103.72	F=30.26
10	36	89.30	
11	36	0.00	

2.2 Effect of slope direction on root nodule nitrogen fixation

Root nodule samples from shady sunny slopes were taken once a month to test the nitrogen fixation enzyme. The results in Table 2 show that, apart from the evident effect of season, rainfall also played an active role in nitrogen fixation. On the shady slope, ANFE continued to drop from June through October. On the sunny slope, the decline was not steady. Statistical analysis showed that the slope direction had no significant effect on ANFE.

Table 2. The effect of slope direction on root nodule nitrogen fixation enzyme

Month	Shady slope		Sunny slope		Rainfall (mm)
	No. of samples	ANFE (n molC_2H_4/(g x min))	No. of samples	ANFE (n molC_2H_4/(g x min))	
6	12	191.4	17	180.9	63.1
7	18	184.0	21	77.5	34.8
8	18	121.9	18	202.8	234.8
9	18	130.8	17	76.6	33.1
10	18	44.2	18	112.1	21.7

2.3 The relation of season change and root nodule nitrogen fixation enzyme of seabuckthorn plants at different ages

Root nodules were sampled from forests with different directions of slope and ages. The results listed in Table 3 and Table 4 show that root nodule nitrogen fixation enzyme in 2-year old plants reached a peak in June and dropped evidently in July. In 6-year old plants, it remained steady from June to August. According to Table 4, nitrogen fixation enzyme in shady slopes was evidently affected by the plant age. The enzyme has also been strongly affected by sampling season.

The annual average values show no significant difference between different ages of plants on sunny slopes. On shady slopes, root nodules of 4 year-old seabuckthorn plants show high enzyme activity.

2.4 The effect of plant sex on ANFE

On shady slopes, the enzyme of the female was more active than that of the male during June and July. Enzymes of female and male plants were equivalent from August to October. Statistical analysis showed that plants' sex had no effect on ANFE.

Table 3. The change of ANFE with the age of plant

Month	2 years		4 years		6 years	
	No. of samples	ANFE (n molC_2H_4/(g x min))	No. of samples	ANFE (n molC_2H_4/(g x min))	No. of samples	ANFE (n molC_2H_4/(g x min))
6	7	235.7	11	195.1	11	143.4
7	12	94.0	13	133.1	12	152.1
8	12	146.1	12	170.9	13	172.5
9	12	117.9	12	109.6	13	84.3
10	12	66.5	12	99.6	12	68.3
11	12	0	12	0	12	0

Table 4. **The change of ANFE with direction of slope and age of plant**
(n molC_2H_4/(g x min))

Month	Shady slope			Sunny slope		
	2 y.	4 y.	6 y.	2 y.	4 y.	6 y.
6	184.01	227.58	149.50	179.26	156.04	138.35
7	150.04	194.99	206.23	45.51	79.89	106.89
8	101.06	120.55	144.12	191.15	221.21	196.99
9	148.39	146.94	97.11	87.38	72.24	69.07
10	4.07	76.51	52.00	128.90	122.76	89.80
Average:	104.36	153.30	129.11	126.44	127.91	122.97
F:	$F_{0.05}$=2.776 F=4.09			$F_{0.05}$=2.776 F=1.07		

2.5 Estimation of nitrogen fixation ability of seabuckthorn root nodule

Age, sex and direction of slope have no clear effect on ANFE. The older plant has more root nodules than the young one and therefore fixes more nitrogen than the young one. In addition, direction of slope and plant density are the other two important factors related to the total nitrogen fixation. According to the investigation in Fangshan County, with a clump density of 2250-3750 clumps per ha, every seabuckthorn plant of 4 years of age has about 50-100g of fresh root nodules. The results of 175 samples showed that the average ANFE of fresh root nodule was 129.7 n mol C_2H_4/(g x min). Further, the nitrogen fixation capacity is 54.14 n mol C_2H_4/(g x min) based on the former value (Shanghai Institute of Plant Physiology 1977). It was estimated that per hectare of seabuckthorn forest of average growth and with a density of 3000 clumps per ha, 29.47-58.94 kg of nitrogen could be fixed every year.

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