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Analysis of Nitrate Reductase Activity for Dominant Tree Leaves in the Northern Aspect Forest of Changbai Mountain, China[1]

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중국 장백산 북사면 산림에서 우세목의 잎 내 질소 환원 효소 활성도 분석[1]

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ABSTRACT

This paper concerned the application of improvement in Vivo of Traditional Method for determination of nitrate reductase (NR) activity of leaves to dominant tree species in five forest communities of northern aspect of Changbai Mountain. The results indicated that the NR activity of tree species was related to shade tolerance, and the intolerant tree species had higher NR activity. The NR of a species was also related to the vertical structure and ecological site condition. The tree species, which have higher NR activities should be selected for fast growing and high yield tree species.

Keywords : *nitrate reductase (NR) activity, shade tolerance, Changbai Mountain, Pinus koraiensis.*

요 약

본 논문은 중국 장백산 북사면의 5가지 산림 군집에서 우세목의 잎 내 질소 환원 효소의 활성도를 보다 개량된 방법을 적용하여 측정 분석한 내용을 다루었다. 질소 환원 효소의 활성도는 수목의 내음성과 연관이 깊은 것으로 파악되어, 양수일수록 질소 환원 효소의 활성도가 높은 것으로 측정되었다. 또한 질소 환원 효소의 활성도는 산림내의 수직적 구조와 생태적 입지 조건과 연관이 깊은 것으로 나타났다. 질소 환원 효소의 활성도가 높은 수종들은 생장이 빠르고 생산력이 높은 것으로 판단된다.

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INTRODUCTION

Nitrogen (N) is one of the most important elements in most forest ecosystems, because it is required for tree growth and nitrogen deficiency is the most common limitation factor for tree growth. Nitrogen has the characteristic of mobility, and only the part of the total nitrogen in the soil can be utilized by plants. It has been noted that tree species differ in their nitrogen requirements, because the preference of nitrogen of a species is mainly dependent upon the soil fertility. It means that some species occur only on fertile soil while others can grow on infertile soil. Stone (1973) cited the early work of Mitchell and Chandler, which divided common deciduous species into three categories with respect to nitrogen requirements. The species that are tolerant of low nitrogen, such as *Quercus prinus*, *Acer rubrum*, and *Populus tremuloides*, have highest competitive ability in the nitrogen poor site. On the other hand, those species of high nitrogen requirements, such as *Fraxinus americana*, *Liriodendron tulipifera*, and *Tilia americana*, are competitive in nitrogen abundant site.

Nitrate, nitrite, ammonium and other nitrides, such as urea, can be used by trees. But most of nitrogen is absorbed in the form of nitrate or ammonia. Many studies have indicated that most of nitrates are reduced into NH_4^+ in leaves and roots after being absorbed by roots. NH_4^+ is further assimilated into organic substances by the enzymes in the chloroplast or the plastids of roots after uptake by trees (Lewis, 1986).

The first step of nitrate assimilation is the reduction of nitrate to nitrite by the enzyme of nitrate reductase (NR). The enzyme is very complex and has a requirement for molybdenum. NR may be present in roots and leaves, although in some plants it appears to be lacking in root. Nitrate is evidently translocated to leaves prior to reduction to nitrite in those plants that lack NR in roots. Once nitrite is formed by reduction through the catalysis of NR, it is rapidly reduced to ammonia by nitrite reductase. Most plants contain only small quantities of nitrite, being a toxic chemical. Likewise, NR activity reflects the capacity and speed on assimilating nitrate (Ting 1982). Air pollutants may stimulate activity of NR. For example, one day after exposure of *Picea rubens* seedling to NO_2 began, NR activity was greatly increased over that in control plants (Norby *et al.*, 1989).

Hageman and Hucklesby (1971) and Hageman and Reed (1980) suggested the method on determination of nitrate reductase activity in leaves. In 1980s, some Chinese researchers successfully determined the NR activity in economic plant leaves and made some improvement for this technique (Zhou and Zheng, 1985). Zhu and Su (1986) in the Chinese Academy of Science, used improved method to determine the NR activity in the economic species and regard it as the biochemical index for deciding on whether a tree species is fast growing and high yield one or not. So studying the NR activity has important theoretical value and practical use.

Table 1. The condition of study forests in the northern aspect of Changbai Mountain.

Sample Forest	Altitude (m)	Cover (%)	Forest type	Species composition(%)			
				Canopy		Regeneration layer	
A	740	50	*P. davidiana-B. platyphylla* forest formed after destruction of broadleaved-*P. koraiensis* forest in conifer-broadleaved mixed forest strip	*P. davidiana* *B. platyphylla* *Tilia amurensis*	60 30 10	*Acer mono* *T. amurensis* *P. koraiensis*	40 30 30
B	740	90	*P. davidiana-B. platyphylla* forest formed after destruction of broadleaved-*P. koraiensis* forest in conifer-broadleaved mixed forest strip	*P. davidiana* *B. platyphylla* others	55 40 5	*A. mono* *T. amurensis* *F. mandshurica* *P. koraiensis* others	30 20 20 20 10
C	740	80	Broadleaved-*P. koraiensis* forest in conifer-broadleaved mixed forest strip	*P. koraiensis* *T. amurensis* *F. mandshurica* *A. mono*	30 30 30 10	*F. mandshurica* *A. mono* *P. koraiensis* *T. amurensis*	50 30 10 10
D	1200	85	*P. koraiensis-Picea asperata* forest in dark conifer strip	*P. koraiensis* *P. asperata* *Abies fabri* others	35 35 20 10	*A. fabri* *P. koraiensis* others	60 30 10
E	1620	93	*B. ermani-P. asperata* forest in dark conifer strip	*P. asperata* *A. fabri* *B. ermani*	60 30 10	*P. asperata* *A. fabri* *B. ermani*	20 20 60

MATERIALS AND METHODS

Study area

We selected the standard trees among the dominant tree species at different elevations in the forest communities of northern aspect of Changbai Mountain, in China. The canopies of standard trees were divided into understory, midstory and overstory. The leaves were taken off from the four directions (east, west, south, and north directions), and mixed up as the samples of analysis. The conditions of forest communities in northern aspect of Changbai Mountains are illustrated in Table 1.

Analysis method

The determination of nitrate reductase (NR) activity was done by using improved method (Zhou Shu et al., 1985). First, the middle part of leaves was cut into small pieces with the length of 1 cm. Then we weighed 1 g and put them into the buffering solution of KNO_3 (50 mM) and H_3PO_4 (0.1 M) as comparing solution. One ml of 1% propyl alcohol was added into the two solutions, which were then put into the vacuum pump to draw the air out of the pump until the leaves were deposited into the solution. Next, we placed the solutions in the dark room in the condition of 25℃ to culture for 30 minutes, took them out of the dark room and put them on the oscillator to vibrate for 2 minutes, sucked up culturing solutions of 5 ml from each of solutions, and added 5 ml gray reagent into them. We used spectrophotometer (model 721, λ=530 nm) to determine the value of comparing color. The determination results were expressed by NO_3^--Nμg(g fw)$^{-1}$(30min)$^{-1}$.

RESULTS AND DISCUSSION

The values of nitrate reductase (NR) activity for dominant tree species in the canopy of broadleaved-*Pinus koraiensis* forest were presented in Table 2. *Populus davidiana* had greatest NR activity among studied tree species, followed by *Betula platyphylla*, *Fraxinus mandshurica*, and so forth. The result indicated that above mentioned three species had higher capacity for absorbing and using NO_3^- than other species. In the original broadleaved-*P. koraiensis* forest, supposed to be climax community, except *F. mandshurica*, the NR activities of *P. koraiensis*, *Tilia amurensis*, and *Acer mono* were small, showing not much differences. It is more likely to note that the NR activity might be associated with the shade tolerance and/or successional stage of tree species. Light demanding tree species with lower climax index, estimated by Kim (1993), had higher NR activity and more capable of absorbing and using NO_3^-. The species of high NR activity belong to nitrogen endurance species group and have high capacity for competing with other species in the nitrogen impoverished soil, prevailing circumstances in disturbed forest. Those species might grow fast so as to play the pioneer role in the disturbed conditions along secondary succession.

The values of NR activity for advance growth under the canopies of broadleaved-*P. koraiensis* forest and *P. davidiana*-*B. platyphylla* forest were shown in Table 3.

Table 2. The NR activity of dominant tree species in the canopy of broadleaved-*P. koraiensis* forest.

Sample Forest	Tree species	The NR activity of leaves NO_3^--Nμg · (g fw)$^{-1}$ · (30min)$^{-1}$
A	*P. davidiana*	5.67
	B. platyphylla	2.10
B	*P. davidiana*	6.30
	B. platyphylla	2.69
	F. mandshurica	4.34
C	*P. koraiensis* (new needles)	0.77
	P. koraiensis (1-yr-old needles)	0.63
	T. amurensis	0.42
	A. mono	0.28

Table 3. The NR activity of regenerating seedlings under the canopies of broadleaved-*P. koraiensis* forest and *P. davidiana-B. platyphylla* forest.

Sample Forest	Tree species	The NR activity of leaves NO_3-Nµg · (g fw)$^{-1}$ · (30min)$^{-1}$
A	*P. koraiensis* (new needles)	1.68
	P. koraiensis (1-yr-old needles)	1.30
	T. amurensis	2.94
	F. manAdshurica	0.70
	A. mono	1.05
B	*P. koraiensis* (new needles)	1.40
	P. koraiensis (1-yr-old needles)	0.42
	T. amurensis	8.10
	F. mandshurica	4.69
	A. mono	0.42
C	*P. koraiensis* (new needles)	0.49
	P. koraiensis (1-yr-old needles)	0.49
	T. amurensis	2.50
	F. mandshurica	0.18
	A. mono	1.26

Most of advance growth that might be established under the canopy of original broadleaved-*P. koraiensis* forest were *Fraxinus mandshurica, Tilia amurensis* and *Acer mono.* The number of *P. koraiensis* seedlings was small. Even though the number of regenerated seedlings of *P. davidiana* and *B. platyphylla* living under the canopy of *P. davidiana-B. platyphylla* forest was also small, we could readily find the seedlings of *P. koraiensis, T. amurensis, F. mandshurica* and *A. mono.*

In the *P. davidiana-B. platyphylla* forest, the regenerated seedlings of *T. amurensis* had highest value of NR activity. Other species, such as *P. koraiensis, F. mandshurica,* and *A. mono* revealed some variation by sample plot. Seedlings of NR activity were comparatively low in the broadleaved-*P. koraiensis* forest, showing that *T. amurensis* was most active in NR, followed by *A. mono, P. koraiensis,* and *F. mandshurica.* Thus these results indicated that different kinds of regenerate seedlings and different individual of a species living in the different ecological environment had different NR activity, which was mainly related to the shade tolerance of species and their ecological conditions.

It is likely that these seedlings showed relatively high capacity for absorbing and using NO_3^-. But they mostly utilize nitrogen in the form of NH_4^+-N with the improvement of the condition of nitrogen, their capacity for competing with *P. davidiana* and *B. platyphylla* becomes higher than before. At last, *P. davidiana-B. platyphylla* forest would be replaced by the

Table 4. The NR activity of leaves in *P. asperata-A. fabri* forest mixed by *P. koraiensis*, sample forest D.

Species	Layer	The NR activity of leaves NO_3-Nμg · (g fw)$^{-1}$ · (30min)$^{-1}$
P. koraiensis	Seedling	1.19
	Understory	2.80
	Mid-story	4.20
	Overstory	4.97
P. asperata	Seedling	1.96
	Understory	Small
	Mid-story	Small
	Overstory	Small
A. fabri	Seedling	Minimum
	Overstory	Minimum

broadleaved-*P. koraiensis* forest. The seedlings of *P. koraiensis* and *T. amurensis* living under the canopy of the original broadleaved-*P. koraiensis* forest compete highly with main canopy above them, which limits their growth. So, the upper canopy contributes to the regeneration of the seedlings. In summary, the NR activity of leaves has some relationship with the regeneration of different species and individuals. This research is important for studying the composition of regenerate tree species in forest community.

The NR activity values of leaves in *P. asperata-A. fabri* forest mixed with *P. koraiensis* by vertical layers were presented in Table 4. The results of experiment showed that only *P. koraiensis* had high NR activity in the multi-storied forest composed by trees having different ages, its NR was different on different stories and increased from understory to overstory. But, the NR activity of seedling in the forest was small. No matter in which layer, the NR activity of *P. asperata* and *A. fabri* leaves was always very small.

Because of the special location of this forest community, the NR activity of dominant trees in this type of forest emerged unusual results. It meant that the NR activity of *P. koraiensis* in this forest community was higher than that in broadleaved-*P. koraiensis* forest. The NR activity of *P. asperata* leaves was much lower than that of *P. asperata* leaves in the *B. ermani-P. asperata-A. fabri* forest. The reason seemed that the *P. koraiensis-P. asperata-A. fabri* forest was not only the upper limit of the distribution of *P. asperata*. The change of species composition and ecological condition made *P. koraiensis* and *P. asperata* absorbing different forms of nitrogen. The absorbing and transforming capacity of NO_3^--N in *P. koraiensis* was improved. While *A. fabri* and *P. asperata* absorb N mainly in the form of NH_4^+-N.

The NR activity values of leaves in *B. ermani-P. asperata-A. fabri* forest and the *B.*

Table 5. The NR activity of leaves in the *B. ermani-P. asperata-A. fabri* forest and the *B. ermani* forest, sample forest E.

Species	Layer	The NR activity of leaves NO_3-Nμg · (g fw)$^{-1}$ · (30min)$^{-1}$
P. asperata	Seedling	1.68
	Understory	4.97
	Mid-story	0.35
	Overstory	0.35
A. fabri	Seedling	Small
	Understory	Small
	Mid-story	Small
	Overstory	Small
B. ermani	Seedling	3.79
	Understory	3.05
	Mid-story	7.15
B. ermani in *B. ermani* forest	Understory	4.76

ermani forest by vertical layers were presented in Table 5. The results of determination showed that the NR activity of *B. ermani* and *P. asperata* was high in the *P. asperata-A. fabri* forest mixed by *B. ermani*. As far as the NR activity in the leaves of subalpine forest was situated between those in mid-story and oversory in the *B. ermani-P. asperata-A. fabri* forest, the NR activity of *B. ermani* was higher than that of *P. asperata* in this forest. There was almost no NR activity in the leaves of *A. fabri*. The capacity for absorbing and utilizing NO_3^--N was highest in *B. ermani,* followed by *P. asperata* and *A. fabri*. It is the high NR activity of *B. ermani* that it occupies the upper limit of forest in Changbai Mountain.

CONCLUSION

The NR activity of leaves was related to the shade tolerance of tree species. The NR activity of shade tolerant species such as *Picea asperata, Tilia amurensis, Abies fabri,* and *Acer mono* was lower than that of shade intolerant, light demanding species such as *Populus davidiana, Betula platyphylla* and *Fraxinus mandshurica*. That means light demanding species have high capacity for absorbing and utilizing NO_3^--N. They also belong to impoverished nitrogen endurance species, which help them firstly occupy the cut over land area and/or the burned site.

The NR activity of leaves was also associated with locations of tree species in the forest community. In the some type of forest, especially in the multi-stories, individuals belonging to the same species had different NR activity in different stories, such as *Pinus koraiensis, P. asperata,* and *B. ermani*. In the *P. asperata-A. fabri* forest mixed by *P. koraiensis,* the NR activity of leaves decreased with the increasing of the height of forest stories.

The NR activity of a species was related to ecological conditions. The NR activity of seedlings of *P. koraiensis* in *P. davidiana-B. platyphylla* forest was bigger than the one in the original broadleaved-*P. koraiensis* forest, while seedlings of *P. koraiensis* living in the *P. davidiana-B. platyphylla* forest having similar environment conditions had similar NR activity.

The tree species of high NR activity such as *P. davidiana, B. platyphylla* had high capacity for absorbing NO_3^--N and enduring the conditions of impoverished nitrogen. They have advantage to compete with other species on the denuded area and belong to pioneer tree species. It is fit to select them for fast growing and high yield tree species. These species should be fertilized by NO_3^--N. *P. koraiensis* belongs to mid-tolerant species and has middle NR activity. It not only has the high ability to utilize NO_3^--N but can absorb NH_4^+-N. Thus *P. koraiensis* should be cultivated in the mature *P. davidiana-B. platyphylla* forest to achieve the aim of planting conifer and protecting broadleaved trees.

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