Influence of the Different NO_3^-/NH_4^+ on mRNA of NR in Sugar Beet

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Abstract: In sugar beet seedling period and sugar accumulation period, the quantity of NR-mRNA raised with the rate of nitrate nitrogen and ammonium nitrogen raised, and the activity of NR raised also, that was the quantity of NR-mRNA of NO_3^-/NH_4^+ —4:0 was the highest. In phyllome formation period and root growth period, the quantity of NR-mRNA of NO_3^-/NH_4^+—3:1 was the highest, and its activity was the highest and that of NO_3^-/NH_4^+—4:0 was the second, that was because these periods were peak of absorbing nitrogen. The results showed that nitrate nitrogen regulated NR in transcriptional level. [Nature and Science 2003;1(1):39-41].

Key words: different NO_3^-/NH_4^+; mRNA of NR; sugar beet

1. Introduction

Nitrate reductase (NR) is a key enzyme involved in the first step of nitrate assimilation in plants, and it is also found in bacteria and fungi. Although in these different organisms it catalyzes a similar reduction of nitrate, the enzyme displays a variety of structures and dissimilarities. In *Escherichia coli* the enzyme is a heterotrimer bound to the bacterial membrane (Calza, 1997) and in *Chlorella* it is anhomotetramer (Howard, 1992) found in the pyrenoid. In plants the enzyme is a homodimer possibly interacting with the chloroplast outer membrane. Apart from NADH, the three other cofactors involved in the reduction of nitrate by NR are FAD, cytochrome b557 and the molybdenum cofactor. The NR from several plant species has been purified to homogeneity (Redinbaugh, 1995) shown to catalyze, apart from nitrate reduction, other reactions, such as the reduction of ferricion (Campbell, 1995), which may be of physiological significance. The subunits of these plants' NR have a molecular weight close to 110 kDa.

The regulation of NR activity in plants appears to be rather complex and many studies have been devoted to the description of this regulation (Huang, 1999). For instance, the catalytic process of nitrate reduction takes place in the leaves of numerous plant species, but it can also occur exclusively in the roots of other species such as white lupin. Molecular tools have been using to study more deeply the features of these regulations, for instance, NR monoclonal antibodies have been available, and the isolation and characterization of a cDNA encoding more than 50% of the tobacco NR mRNA is presented. For the basis of studying sugar beet NR mRNA, the methods of Northern and Southern are complex, and the polymerase chain reaction (PCR) technology is simple and it can analyze the micro-DNA. In this study we used PCR technology to quantitatively analyze NR mRNA of sugar beet that controlled by different nitrogen source. We engineered NR genes to improve utilization rate of nitrogen fertilizer and culture top quality and high yield sugar beet in practice.

2. Materials and Methods

***Materials*:** The present leading variety in Heilongjiang Province of China — Tian Yan 7 was used in this study.

***Plant Culture*:** The gravel culture was used and the nutrient solution was improving Hoagland. A nutrient solution containing mixed nitrogen-ammonium nitrogen and nitrate nitrogen (total nitrogen source is 8.0 mmol/L) was used. The nitrogen were used as the different rate: NO_3^-/NH_4^+ 4:0, 3:1, 2:2, 1:3, 0:4, respectively. The nutrient solution was changed every 7 days and regulated the pH every 3 days to keep it neutrality. From seedling stage sampling was done from 8 to 10 o'clock in sunny morning per 20 days. Functional leaves were sampled to measure NR activity.

***Measurement of NR*:** Sulfanilamide colorimetric method.

***Quantity of NR-mRNA*:** Extracted total RNA (according to a procedure of Trizol kit) and got intact RNA → purified RNA → inverse transcription (according to a procedure of Promega inverse transcription kit, first strand cDNA was synthesized from total RNA using primers P_1 5'GAA CAC AGC TTC CAA GAT CAT CCA 3', P_2 3'GTTATGATGTCACCAATATACCCT 5') → PCR [with 40 cycle of 94℃ (30') denaturation, 60℃ (1') annealing, 72℃ (2') extension]. PCR products were resolved in 2% agarose gels and were visualized by ethidium bromide staining, then scanned by CS-930 chromato-scanner (made in Japan).

3. Results

Extraction and Selection of Total RNA from Sugar Beet: Total RNA was extracted from sugar beet leaf tissues according to a procedure in Trizol kit. The sample was determined in λ_{260nm}, λ_{280nm}, λ_{230nm} differently (Table 1). The results showed that OD_{260}/OD_{280} of RNA was 1.932 and it was in the middle of 1.8- 2.0. Electrophoresis was carried out. The electrophoretogram (Figure 1) showed that the products 23sRNA, 28sRNA and 5sRNA were obtained. It was intact and it could be done RT-PCR (reverse transcription- polymerase chain reaction).

Table 1. Quality Determination of RNA

OD	OD_{260}	OD_{280}	OD_{230}	OD_{260}/OD_{280}	OD_{260}/OD_{230}
RNA	0.398	0.206	0.197	1.932	—

Figure 1. Electrophoretogram of Sugar Beet Total RNA

Table 2. The Effect of Different NO^-_3/ NH^+_4 on the Activity of NR in Endogenoussubstrate (μ gNO^-_2/gFWh)

Control NO_3^-/NH_4^+	Date of Sampling						
	6.14	6.25	7.15	8.01	8.17	9.13	9.25
CK	0.446	4.057	8.728	1.424	4.289	2.673	1.879
4:0	2.101	5.779	16.134	1.784	6.097	4.105	2.348
3:1	1.734	6.893	17.556	2.026	7.381	4.029	2.189
2:2	1.619	5.274	15.931	1.706	5.788	4.096	2.102
1:3	0.904	4.969	11.642	1.498	4.403	3.028	1.884
0:4	0.716	3.986	10.958	1.349	4.039	2.952	1.904

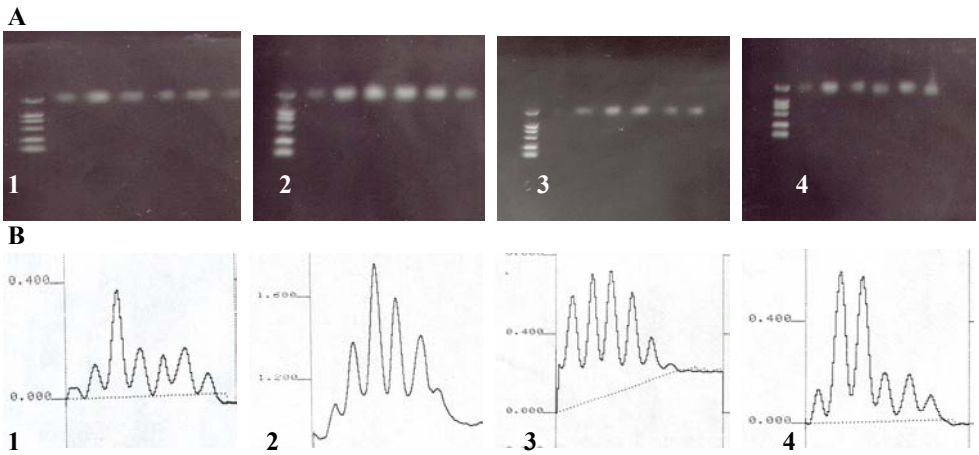

Figure 2. Quantity of NR-mRNA from Sugar Beet in Different Growth Period. (A1-4) Electrophoretogram of RT-PCR product on NR-mRNA. The bands were mark, ck, 4:0, 3:1 ,2:2, 1:3, 0:4 in proper order from left to right. (B1-4) chromatogram of electrophoretogram of RT-PCR product on NR-mRNA. (1) seedling period. (2) phyllome formation period. (3) sugar accumulation period. (4) root growth period.

The Measurement of NR Activity and Quantity of NR-mRNA from Sugar Beet Leaves. In sugar beet seedling period and sugar accumulation period, the quantity of NR-mRNA raised with the rate of nitrate nitrogen in mixed nitrogen sources raised, and the activity of NR raised also. In the quantity of NR-mRNA of NO_3^-/NH_4^+—4:0 was the highest. In phyllome formation period and root growth period**,** the quantity of NR-mRNA of NO_3^-/NH_4^+—3:1 was the highest, and its activity was the highest and that of NO_3^-/NH_4^+—4:0 was the second. This showed that adding small amount of NH_4^+-N under the circumstance of NO_3^-–N was abundant to improve the NR-mRNA transcriptional quantity and raise the NR activity same time. But in every growth period with the rate of ammonium nitrogen in mixed nitrogen sources raised the quantity of NR-mRNA and NR activity was decreased deeply (Table 2, Figure 2). We know that NH_4^+-N could inhibit the NR activity, but in the periods of absorbing nitrogen peak, NH_4^+-N could stimulate the NR-mRNA also. The results showed that the NR activity was controlled at the transcriptional level and nitrate nitrogen regulated NR at the transcriptional level.

4. Discussion

We know that NO_3^-–N could induce the NR activity, but in period of uptaken nitrogen peak of sugar beet – that is NH_4^+-N could improve NR activity also. Because this was in the period of uptaken nitrogen peak, NH_4^+-N could improve the utilization rate of NO_3^--N and improve transcriptional quantity of NR-mRNA. The rate of NH_4^+-N was increased in mixed nitrogen source, and the NR-mRNA level was decreased. NH_4^+-N can stimulate the GS activity and improve Gln. But, as the reference showed (Xiao, 2000) a negative correlation between NR-mRNA level and increasing Gln, and the NR-mRNA level decreased sharply when Gln was improved to plants. This paper showed that we could control NR activity of sugar beet at the transcriptional level. So, we may engineer NR genes by molecular biological technology to improve NR-mRNA level and increase NR activity. We could improve utilization rate of nitrogen fertilizer and culture top quality and high yield sugar beet in practice.

The financial support provided by the National Natural Science Fund (39770434).

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