Journal of Animal and Veterinary Advances 11 (17): 3111-3118, 2012
ISSN: 1680-5593

The Effects of Nitrogen Fertilizer or Low Temperature on the Nitro-Compound of Corn

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Abstract: The nitro-compound, nutritive material and silages fermentation of corn were studied under different nitrogen fertilizer application or temperature, the results showed that corn had good fermentation quality under different nitrogen fertilizer application or temperature. The DM (Dry Matter), CP (Crude Protein) contents of corn were increased significantly at the 0.05 level with the increased of fertilizer nitrogen amount however, the WSC (Water Soluble Carbohydrate) content decreased but these were eliminated by low temperature treatment. And the nitrogen content increased significantly at the 0.05 level under different nitrogen fertilizer application or temperature. The nitrate content reduced post-ensilaged and the nitrite content improved significantly, respectively. The enzymatic activities of NR (Nitrate Reductase) and PM H^+-ATPase (Plasma Membrane H^+-ATPase) were improved significantly by the use of nitrogen fertilizer treatment although, they were reduced under low temperature. The nitrogen contents in the soil decreased after planted. However, it was improved under different nitrogen fertilizer application or temperature, the coefficient of soil nitrogen utilization of corn were not changed significantly even the nitrogen content improved under low temperature treatment.

Key words: Nitrogen fertilizer, low temperature, corn, silages fermentation, nitro-compound

INTRODUCTION

Corn (*Zea mays* L.) originated in Mexico and Peru of South America. Gramineous corn is an annual herb which is the world's most widely distributed crop. As an important food and feed crops, corn ensiled for silage can improve labor efficiency and labor saving and which strengthens the utilization of the nutrients including nitrogen, phosphorus and potassium. Applying nitrogen fertilizer is one of the effective measures to improve plant production and a key factor in forming forage quality (Muraoka *et al*., 2009). Besides it will improve plant biomass and simultaneously cause the rise of the nitrogen content of the soil and the enrichment in nitro compounds of plant (Lawlor, 2002). There are almost no studies suggesting that the accumulation of nitrate *in vivo* of the plant is closely related to its Nitrate Transporting System. Two nitrate transporting systems are existing in plant and are respectively regulated by the high affinity and low affinity nitrate transport proteins on the membrane (Glass *et al*., 2002) both of which are related to the active transport of H^+-ATPase across the plasma membrane (Miller and Smith, 1996). As one of important environmental factors, temperature plays a vital role in plant growth and development process and low temperature stress adversely affects various physiological activities of plants (Su *et al*., 1990).

H^+-ATPase of the plasma membrane and tonoplast can take control of intracellular pH, resulting in the electrochemical potential gradients and enhance ion and molecular transport and other important physiological functions in addition, both of them show a high sensitivity and plasticity when exposed to cold stress and their activity variation are considered to be the initial physiological response as the plant cell suffers low temperature stress (Kasamo, 1988; Moriyama and Nelson, 1989).

In most parts of Northern of China, the low temperature is a main obstacle what most plants encounter in the process of growth and development. Under low temperature conditions, there is a decline in the biomass and quality of the plant and variation in

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physiological and biochemical changes including changes in activity of many enzymes. Many articles having been published interiorly reported about the effects of nitrogen fertilizer and low temperature stress on plants including a lot of researches on corn silage but few on sweet sorghum silage and pearl millet silage and no studies on eliminate nitrate enrichment by the means of making silage.

MATERIALS AND METHODS

Experimental design: The nitro-compound, nutritive material and silages fermentation of Nongda 86 which is a breed of corn silage from China Agriculture University were studied under different nitrogen fertilizer application or temperature stimulus.

Plastic drums (inner diameter 22 cm, height 36 cm) were filled to 7 kg with 30 cm surface soil of testing field from Shanxi Agricultural University and put in greenhouse in November 2009. The saturated soil water content remained at 80% according to the plastic drums' height after seeding, corn were treated during the jointing stage with the gradient urea (46% N) treatments as follows: N0: 0 kg/hm^2, N90: 90 kg/hm^2 (1.48 g urea needed per plastic drum), N180: 180 kg/hm^2 (2.95 g urea needed per plastic drum) and N270: 270 kg/hm^2 (4.43 g urea needed per plastic drum) and were under the low temperature environment during the milk stage between 17:00-8:00 next day (The average temperature of out-greenhouse at this time frame has been determined in 0-5°C).

Sampling and sample analysis: The ground plant of all the experiment were cut to 1-2 cm after low temperature stimulus, 10 g of them were put in -80°C ultra cold storage freezer for measuring indices included NR and PM H^+-ATPase, NR was assayed as described by Long and Oaks (1990). The activity of PM H^+-ATPase was assayed according to Zhang *et al.* (2006)'s Method.

Half of the rest were used for ensiling, others was toasted 48 h in 65°C for moisture determination (Vagnoni and Broderick, 1997) and be broke up to 40 mesh size for determining DM, CP, NDF (Neutral Detergent Fiber), ADF (Acid Detergent Fiber), Ash, nitrate and nitrite contents, WSC (Owens *et al.*, 1999). CP was calculated as Kjeldahl N×6.25 (AOAC, 1990, method 954.01). The contents of NDF and ADF were determined according to the methods of Van Soest *et al.* (1991). The ash content was determined by combustion at 600°C for 2 h (AOAC, 1990, method 942.05). The nitrite content was determined using anodic voltammetry described by Santosa *et al.* (2009). The nitrate content was measured according to the methods of Nam *et al.* (2008).

After 90 days ensiling, the pH, ammonia nitrogen (Broderick and Kang, 1980), LA (Lactic Acid), PA (Propionic Acid), BA (Butyric Acid), AA (Acetic Acid) (Zhang and Kumai, 2000) and indexes preceding were still determined. The 0-30 cm soil were taken from all plastic drums by soil auger before seeding and after sampling were killed out 0.5 h in 120°C then were toasted above 8 h to constant weight in 105°C, grinded to 100 mesh size for determining N with UDK132 semiautomatic determination of nitrogen analyzer by Kjeldahl procedure (Bremner and Mulvaney, 1982).

Statistical analysis: Chemical, fermentation, enzymatic activities and soil nitrogen data among different treatments were assessed using the one-way ANOVA procedure of the Statistical Analysis System (SAS 9.1, SAS Institute Inc., Cary, NC, USA). In order to determine whether ensiling differed significantly, t-tests were performed. Treatment differences were considered to be significant when $p<0.05$ and extremely significant when $p<0.01$. The differences among treatment means were tested with the Duncan's multiple range test. Data were expressed as means±SEM.

RESULTS

The fermentation quality of corn silage under different nitrogen fertilization or low temperature stimulus: The pH and ammonia nitrogen of corn silage were increasing gradually with the gradient urea treatments however, indistinctively to the maximums, 3.65 and 5.75%, respectively (Table 1). The pH and ammonia nitrogen of corn silage which were under low temperature stimulus were inconspicuously higher than what under normal temperature to the maximums, 3.96 and 5.82%, respectively. There was no difference in LA, AA and PA of corn silage under different nitrogen fertilizer application or temperature stimulus however, PA was in very small amounts and BA was no found.

The nutritive variation of corn silage under different nitrogen fertilization or low temperature stimulus: The DM content was increased at the 0.05 level and the CP was increased significantly at the 0.01 level, respectively under the gradient urea application while were reduced significantly at the 0.01 level under low temperature stimulus from Table 2. The Ash and WSC contents were no difference under the gradient urea treatments but were reduced at the 0.05 level under low temperature stimulus, however, there were no differences in NDF and ADF contents under the gradient urea application or

Table 1: The fermentation quality of corn silage under different nitrogen fertilization or low temperature stimulus

Nitrogen application rate	Normal temperature 0	90	180	270	Low temperature 0	90	180	270	Significance of effects: Temperature	N fertilization	Interaction
pH	3.32±0.30^{B}	3.48±0.31^{AB}	3.51±0.32^{AB}	3.65±0.42^{AB}	3.57±0.35^{AB}	3.68±0.37^{AB}	3.82±0.41^{AB}	3.96±0.42^{A}	NS	NS	NS
Organic acid/(g*/(100 g)											
LA	4.13±0.41	4.08±0.50	4.01±0.32	3.98±0.28	4.11±0.28	4.05±0.36	3.98±0.31	3.97±0.27	NS	NS	NS
AA	1.20±0.21	1.32±0.28	1.28±0.24	1.25±0.18	1.14±0.11	1.19±0.16	1.21±0.10	1.15±0.09	NS	NS	NS
PA	0	0	0	0	0.01	0	0	0.02			
BA	0	0	0	0	0	0	0	0			
Ammonia nitrogen/TN%	4.56±0.87	5.52±1.14	5.75±1.07	5.70±1.21	4.67±0.98	5.58±1.01	5.82±0.96	5.73±0.91	NS	NS	NS

Table 2: The nutritive variation of corn under different nitrogen fertilization or low temperature stimulus

Nitrogen application rate	Normal temperature 0	90	180	270	Low temperature 0	90	180	270	Significance of effects: Temp.	N fertilization	Interaction
DM (%)	27.51±1.34^{Bb}	29.61±1.28^{ABab}	31.68±1.34^{Aa}	31.69±1.05^{Aa}	27.30±1.12^{Bb}	7.21±0.44^{Dd}	8.35±0.32^{Cbc}	9.11±0.28^{Bb}	**	**	**
CP (DM %)	6.17±0.57^{Ee}	8.17±0.36^{Cc}	10.07±0.11^{Aa}	10.45±0.28^{Aa}	6.02±0.24^{Ee}	7.21±0.44^{Dd}	8.35±0.32^{Cbc}	9.11±0.28^{Bb}	**	**	**
NDF (DM %)	56.01±1.94	55.89±1.25	55.78±1.4	56.08±1.57	55.94±1.25	55.82±1.08	55.39±1.65	55.87±1.26	NS	NS	NS
ADF (DM %)	30.64±1.24	30.49±1.05	30.55±1.54	30.67±1.24	30.56±1.32	29.44±1.25	29.87±1.60	30.24±1.32	NS	NS	NS
Ash (DM %)	5.97±0.24^{A}	5.98±0.18^{A}	5.97±0.36^{A}	6.01±0.24^{A}	5.86±0.15^{A}	5.84±0.12^{A}	5.57±0.11^{B}	5.92±0.14^{A}	*	NS	NS
WSC (DM %)	7.19±0.18^{Bb}	7.11±0.24^{Bb}	7.08±0.17^{Bb}	7.01±0.21^{Bb}	7.55±0.10^{Aab}	7.61±0.14^{Aa}	7.55±0.08^{Aab}	7.32±0.20^{ABab}	**	NS	NS
Nitrate ($mg\ kg^{-1}$ DM)	876.79±63.12^{Fe}	938.17±38.28^{Ede}	1034.64±39.86^{CDcd}	1205.38±64.05^{Bb}	942.37±38.24^{Ede}	987.64±42.14^{DEcde}	1103.24±28.66^{Cbc}	1328.75±68.32^{Aa}	**	**	NS
Nitrite ($mg\ kg^{-1}$ DM)	1.14±0.53^{Bb}	1.54±0.28^{ABab}	1.81±0.34^{Aab}	2.01±0.27^{Aa}	1.18±0.24^{Bb}	1.25±0.05^{Bb}	1.72±0.13^{ABab}	1.86±0.17^{Aab}	NS	**	NS

Table 3: The nutritive variation of corn silage under different nitrogen fertilization or low temperature stimulus

Nitrogen application rate	Normal temperature 0	90	180	270	Low temperature 0	90	180	270	Significance of effects: Temp.	N fertilization	Interaction
DM (%)	27.98±1.21	28.37±1.31	28.24±1.08	28.26±1.2	27.34±1.32	28.25±1.22	28.19±1.30	28.21±1.21	NS	NS	NS
CP (DM %)	6.09±0.24^{Ee}	7.95±0.27^{Cc}	9.17±0.21^{Bb}	9.81±0.17^{Aa}	5.95±0.16^{Ee}	7.18±0.27^{Dd}	8.32±0.29^{Cc}	9.01±0.18^{Bb}	**	**	NS
NDF (DM %)	56.78±1.81	56.25±1.34	56.48±1.58	56.85±1.34	55.32±2.14	55.04±1.64	55.18±1.82	55.26±1.14	NS	NS	NS
ADF (DM %)	31.37±1.57	31.01±1.82	32.17±1.17	30.91±1.08	30.43±1.20	30.58±1.46	30.14±1.23	30.57±1.18	NS	NS	NS
Ash (DM %)	5.91±0.14^{Aa}	5.82±0.23^{Aab}	5.74±0.28^{ABab}	5.92±0.17^{Aa}	5.82±0.06^{Aab}	5.81±0.14^{Aab}	5.51±0.07^{Bb}	5.83±0.10^{Aab}	NS	NS	NS
WSC (DM %)	2.35±0.18^{ABa}	2.41±0.21^{Aa}	2.04±0.17^{Ba}	2.15±0.23^{ABa}	2.34±0.21^{ABa}	2.32±0.13^{ABa}	2.41±0.17^{ABa}	2.33±0.14^{ABa}	NS	NS	NS
Nitrate ($mg\ kg^{-1}$ DM)	358.34±78.01^{Ed}	454.27±62.31^{DEcd}	502.21±48.97^{CDbc}	684.92±63.51^{Aa}	507±65.67^{BCbc}	581.24±36.85^{ABabc}	611.23±45.32^{ABab}	698.54±36.44^{Aa}	**	**	NS
Nitrite ($mg\ kg^{-1}$ DM)	2.21±0.23^{Dc}	2.51±0.34B^{CDabc}	2.92±0.28^{Aa}	2.81±0.36^{ABab}	2.34±0.12^{CDbc}	2.68±0.09^{ABCabc}	2.84±0.14^{ABab}	2.88±0.10^{ABab}	NS	**	NS

Table 4: The enzymatic activities of corn under different nitrogen fertilization or low temperature stimulus

Nitrogen application rate	Normal temperature 0	90	180	270	Low temperature 0	90	180	270	Significance of effects: Temp.	N fertilization	Interaction
NR (μg/g.h)	5.75±0.03^{Dd}	6.12±0.04^{Cc}	6.96±0.03^{Aa}	6.53±0.05^{Bb}	4.36±0.02^{Hh}	4.66±0.01^{Gg}	4.78±0.03^{Ff}	5.01±0.01^{Ee}	**	**	**
PM H^+-ATPase (μg $Pimg^{-1}$ $Pr.h^{-1}$)	36.14±0.12^{Ff}	42.85±0.23^{Cc}	56.31±0.18^{Aa}	46.11±0.24^{Bb}	28.75±0.11^{Gg}	32.03±0.18^{Hh}	37.14±0.12^{Ee}	38.91±0.13^{Dd}	**	**	**

NS: No Significance; ** $p<0.01$; * $p<0.05$; little letters $p<0.05$. Different capital letters in the same row are significant at the 0.05 level, lowercase letters are significantly different at the 0.01 level

temperature stimulus and the interaction effect existed in CP content significantly at the 0.01 level from Table 2. After ensiling, the CP content of silage was increased at the 0.05 level and the WSC was reduced significantly at the 0.01 level, respectively however, the DM, NDF, ADF, ash were no significant changed from Table 2 and 3 by t-test.

The nitro-compound of corn silage under different nitrogen fertilization or low temperature stimulus: The nitrate content was significantly increased at the 0.01 level in corn under the gradient urea application or temperature stimulus; however the nitrite content was significantly increased at the 0.01 level under the gradient urea treatments but no difference with low temperature stimulus (Table 2). After ensiling, the nitrate content reduced post-ensilaged and the nitrite content improved significantly at the 0.01 level, respectively (Table 2 and 3).

The enzymatic activities of corn under different nitrogen fertilization or low temperature stimulus: The enzymatic activities of NR and PM H^+-ATPase were improved significantly at the 0.01 level by the use of nitrogen fertilizer treatment, although they were reduced under low temperature stimulus from Table 4.

Soil nitrogen content after planted corn increased significantly at the 0.01 level under the gradient urea application but changed no difference of normal and low temperature from Table 5.

Table 5: Soil nitrogen content after planted under different nitrogen fertilization or low temperature stimulus (g kg^{-1})

Soil nitrogen content before planted nitrogen application rate	Normal temperature				Low temperature				Significance of effects		
	0	90	180	270	0	90	180	270	Temp.	N fertilization	Interaction
0.0788 ± 0.012^{Aab}	0.0406 ± 0.009^{B}	0.0502 ± 0.008^{Bc}	0.0570 ± 0.011^{Bbc}	0.0805 ± 0.008^{Aab}	0.0415 ± 0.014^{Bc}	0.0517 ± 0.008^{Bc}	0.0581 ± 0.012^{Babc}	0.0823 ± 0.014^{Aa}	NS	**	NS

NS: No Significance; **p<0.01; *p<0.05; Little letters p<0.05. Different capital letters in the same row are significant at the 0.05 level, lowercase letters are significantly different at the 0.01 level

DISCUSSION

Effect of different nitrogen application rate and low temperatures on the fermentation quality of corn silage: The pH is an important indicator which reflects whether it is successful or not for the conventional silage. During the ensiling process if the main growth of lactic acid bacteria dominated, the pH value will drop. And the pH will easily rise if the butyric acid bacteria or spoilage bacteria in the dominant place during the fermentation. Generally, it is good for silage's quality if the pH is lower. Corn owes high carbohydrates and low buffer value and the buffer value of the corn group with the treat of NO was 179.96±6.13 mE kg^{-1} DM in this study which is good for fermentation of lactic acid bacteria. As the results showed, the pH of corn silage increased gradually under different nitrogen application, during which the maximum was 3.65. Besides with the treatment of low temperature, corn silage's pH was higher than those without but there were no significant difference which was all <4.0 and among which the highest pH reached 3.96. Therefore, the fermentation quality of corn silage was excellent.

Silage was ensiled by fermentation of lactic acid bacteria which made the pH dropt quickly and maintained the anaerobic environment in order to make the silage crops a long-term preservation. Therefore, the level of lactic acid content of the silage had a direct impact on the fermentation quality of silage. Either adding nitrogen fertilizer or stimulating of the low-temperature lactic acid content of the silage was about 4.0 and the differences among the test groups were not significant, besides, the content of butyric and propionic acid that has an adverse effects on silage fermentation quality were much less.

Ammonia nitrogen in the silage was the main indicator to assess the quality of silage. It not only reflected how much the protein and amino acid were broken down during the ensiling process but also were closely related to the nutritive value of silage. The higher the ammonia nitrogen content, the more the decomposition of proteins and amino acids and the worse the silage quality. The results showed that the content of ammonia nitrogen in the corn silage gradually increased with increasing amount of adding nitrogen among which the highest content was 5.75% and there was no significant difference. However, the ammonia nitrogen content of corn silage under low temperatures was higher than those without and the maximum was 5.82% in which the changes were not significant. Therefore, application of nitrogen fertilizer and low temperature are effective to have a certain influence on the decomposition of protein and amino acids in the ensiling process but in the view of the absolute content the proportion of ammonia nitrogen were <10% of the total nitrogen which had little effect on silage quality and thus met the requirements of good silage.

In the study, the degradation of the protein and amino acids during the ensiling process was much lower in the room temperature and its feeding value was relatively higher.

Effects of different nitrogen application rate and low temperatures on the nutrient content of corn silage before and after ensiling: Nitrogen was one of the factors for plant in the largest demand and also a general element lack in the soil. Corn which belongs to gramineaes did not have the nitrogen fixing capacity and the nitrogen that needed for its growth and development relied mainly on those absorbed by the roots from the soil (Rhykerd and Noller, 1973). Nitrogen application can promote the synthesis of plant proteins, increased the synthesis of chlorophyll and enzymes related to photosynthesis and enhanced photosynthesis and the accumulation of photosynthetic products which contributed to the vegetative growth of the plant. Simultaneously, adding nitrogen can also improve crude protein, crude fat and amino acid content and lower crude fiber content in the forage crops, improving the quality of the forage crops (Mason and Croz-Mason, 2002; Rasheed and Mahmood, 2004). By adding nitrogen, the total nitrogen, protein nitrogen, non-protein nitrogen, crude protein, true protein, amino acids and nitrate content in plant have increased in somewhat. The spike number, grain yield, protein content of grain and lysine content were all decreased by putting on nitrogen application (Graham *et al*., 1983). Under normal and low temperatures conditions in this study, DM content of corn increased as the amount of nitrogen increased. However, there was no significant difference under normal temperature conditions. On the basis of reasonable phosphorus and potassium, increasing the amount of nitrogen fertilizer could enhanced the protein content which had greatly improved the quality of the corn silage.

The results of this trial showed that CP content of corn increased as nitrogen rate went up and there were significant differences among the treatments indicating that nitrogen can contribute to the protein of corn. Within a certain range, the protein content can significantly increased along with the increasing of nitrogen fertilizer; there was no significant difference between treatments of N180 and N270 which made it clear that the nitrogen supplied for the plant had exceeded the optimal demand. Even though increased the amount of nitrogen fertilizer after the treatment of N270, there would not be an increased in nitrogen needs of the corn and it had caused the accumulation of nitro compounds of corn *in vivo* and in soil under low temperature stress. The differences were significant among the treatments of N0, N90, N180 and N270 indicating that low temperature stress on the corn had caused some impact on its utilization of nitrogen fertilizer and had improved the nitrogen needs of the corn. There were no significant differences among the content of NDF, ADF, ash and WSC of the corn as the nitrogen rate changed indicating that nitrogen fertilizer had no effect on NDF, ADF and WSC. In the growth and development process of plant, it was an inevitable stress of low temperatures and it was the most sensitive physiological processes that the growth of the plant is inhibited by the influence of low temperatures. Photosynthesis is the foundation of a biological product therefore, it is one of the most obvious physiological processes affected obviously by low temperature influence (Berry and Bjorkmann, 1980). As the results showed that under the same nitrogen rate, low temperatures made the content of DM, CP, ADF and ash of corn reduce significantly but had no significant influence on the content of NDF which could explained that low temperatures reduced the growth rate of DM and the synthesis of CP that was consistent. Besides, low temperature stress had an impact on photosynthetic characteristics, making leaf chlorophyll content, the activity of enzyme related to photosynthesis and carbon assimilation decreased, resulting in photosynthetic rate lowered and the composition of the nutrient content reduced.

Contrarily, WSC content was increased under low temperature stimulation at the same level of nitrogen application and the differences were significant indicating that low temperatures could significantly promote the synthesis of WSC (Kiyosue *et al*., 1996). Soluble sugar acting as the osmoprotectant is the main factor causing the change in the total solute concentration of the plant. At low temperatures, the accumulation of soluble sugar increased in plant body and cell concentration increased which contribute to the cold hardiness of plant. The level of soluble sugar content could be used as an indicator of cold resistance for measuring different varieties of wheat. As the temperature and stress time decrease, the result obtained that the content of soluble sugar was increased in varying degrees.

Effects of different nitrogen application rate and low temperatures on the content of the nitro compounds of corn silage before and after ensiling: Many green grass, leaves and fodder crops, contain nitrates in varying degrees. Nitrate contained in the forage had little toxicity on livestock but when it was converted to nitrite, its toxicity increases highly which could result in livestock poisoned. Once the ruminant intake forages which was made up unreasonably and contains too much nitrate, it would be transformed into nitrite by the rumen microbes, resulting in significant accumulation.

Within a certain range of nitrogen with the increase in nitrogen application, the accumulating ability of nitrate in vegetables could be measured by the total cumulative nitrate per plant. Nitrogen needed for plant growth comes mainly from the soil and variation in nitrogen accumulating is highly related to its supplying level which reflected the nutritional state of the plant itself and nutrient requirements (Steingrover *et al*., 1986; Chapin *et al*., 1987). The results showed that content of nitrate in the corn increased significantly as the nitrogen application rate went up under the normal temperature or low temperature conditions with the highest content in the N270 treatment indicating that at high level of nitrogen, absorption intensity of nitrate increased while the restoring capacity was diminished, resulting in the accumulation of nitrate. The addition of nitrogen fertilizer to plants made it much stronger than the growing amount of nitrate needed in growth for the rising accumulation of nitrate which caused the enrichment of nutrient and this was main reason why the nitrate content was positively related to the nitrogen application rate.

Nitrate itself has no toxic effect on the body but that accumulated while not utilized in plants would be transformed into nitrite that can connect with the level amine to form carcinogens-nitrosamines which would result in cancer of the digestive system by the NR. In this experiment, under normal temperature conditions, nitrate in corn increased with the nitrogen application rate which explains that due to the increased amount of nitrogen, the plant not only enriched a lot of nitrate but also some of the reductase reduced to nitrite, causing the content of nitrite in the body of the corn increased significantly either.

At the same level of nitrogen application, the nitrate content of corn was significantly increased by low temperatures with significant difference while no significant difference in nitrate content. Influenced by low temperature, the ability to use nitrogen went down for the plant while the growth rate of it has lagged far behind the speed of the absorption of nitrogen, making the enrichment of nitrate. At the same time, the activity of enzyme related to the nitrate reduction decreased, resulting in nitrate in plants hard to be converted to nitrite and large enrichment of nitrate.

The results showed that corn ensiled, the nitrate of corn silage had significantly decreased while the content of nitrite significantly increased.

Effects of different nitrogen application rate and low temperatures on enzymes activities *in vivo* of corn silage: As a key enzyme of nitrogen metabolism, NR also played a key role in the process of taking and utilizing nitrogen. That NR in the leaves and roots were both higher than any other parts of the corn determined the high metabolic rate of nitrate which made it sure that the metabolism of carbon and nitrogen efficiently went on at the later growth stage (Kaiser and Huber, 1989; Subhash and Leonard, 1987; Eilrich and Hageman, 1973). As the results showed: the activity of corn NR under normal temperature conditions trended to increase at first and then decrease as the amount of nitrogen increase with significant differences in each nitrogen treatment and the order of the active strength was N180>N270>N90>N0 but under low temperatures conditions, it was an upward trend for the NR activity with the increasing nitrogen to which the NR activity was positively related and the more the nitrogen applied, the higher it did but remained when the rate reaches a certain level due to the highest activity itself however, there was no decline in the nitrate content of the corn *in vivo* which was caused by the rising activity of NR because under the high amount of nitrogen application, root's absorbing capacity of nitrate is much stronger than the reduction ability of NR. And what just had said above was consistent with that argued. Under low temperature, the activity of NR decreased while it was promoted and maintained an upward trend as a result of nitrogen fertilizer.

Plasma membrane was the key structure for cell to contact the living environment which not only do regulate all the nutrients to come in or out but also was the first barrier of the cell response to external adverse factors. PM H^+-ATPase was an important enzyme dominating the living process of the plant and plays a regulating role in many important physiological processes of the plant such as transporting nutrients or ions, cell elongation, anisotropic growth, stomata movement, osmotic adjustment and so on (Sen and Osborne, 1974).

As the results showed that under normal temperature the activity of PM H^+-ATPase of corn *in vivo* started to increase and then declined as the amount of nitrogen increased but on the rise at low temperatures which was consistent with the varying trend of the NR for that adequate supply of nitrogen promoted plant growth and enzyme activity and improved the speed of the metabolism. Therefore, the synchronous change in the activity of enzyme promoted the synthesis of nutrients required for the rapid growth of maize and weakened the accumulation of nitrate to ensure the normal growth of plants.

The activity of NR and PM H^+-ATPase of the corn were declined under low temperatures at the same level of nitrogen application with significant differences among the treatment groups. The main reason why the activity of NR and PM H^+-ATPase slowed down were that cold temperatures had a negative influence on the metabolic rate of the plants and that low temperature stress damaged the membrane system. Palta and Li (1978) had speculated that the plasma membrane ATP enzyme may be the original point of freezing injury. PM H^+-ATPase was very sensitive to low temperatures by having conducted experiments on tomato seedlings with cold stress and Winter wheat seedlings with freezing stress. This study revealed the fact that the activity of PM H^+-ATPase of the corn significantly declined under low temperatures.

CONCLUSION

Corn silage had good quality of fermentation under different nitrogen fertilizer application or temperature. The DM, CP contents of corn were increased significantly on 0.05 level with the increased of fertilizer nitrogen concentration however, the WSC content decreased but these were eliminated by low temperature treatment. And the nitrogen content increased significantly on 0.05 level under different nitrogen fertilizer application or temperature. The nitrate content reduced post-ensilaged and the nitrite content improved significantly, respectively. The enzymatic activities of NR and PM H^+-ATPase were improved significantly by the use of nitrogen fertilizer treatment although, they were reduced under low temperature.

The nitrogen contents in the soil decreased after planted. However, it was improved under different nitrogen fertilizer application or temperature, the coefficient of soil nitrogen utilization of corn were not changed significantly even the nitrogen content improved under low temperature treatment.

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