Advance Journal of Food Science and Technology 5(6): 738-742, 2013
ISSN: 2042-4868; e-ISSN: 2042-4876
© Maxwell Scientific Organization, 2013
Submitted: January 31, 2013 Accepted: February 25, 2013 Published: June 05, 2013

Effect of Nitrogen Application Rate on the Enzyme Activity of Flag Leaf after Anthesis in Winter Wheat

Liyuan Yan and Yan Shi
Dryland Technology Key Laboratory of Shandong Province in Qingdao Agricultural University, Qingdao, Shandong, China, 266109

Abstract: This experiment studied the effect of nitrogen application rate on the enzyme activity of flag leaf after anthesis in winter wheat whose results showed that when the nitrogen application was within the range of 0~300 kg/hm^2, with the increased amount of nitrogen, soluble protein content, the activity of Nitrate Reductase (NR) and the Glutamine Synthetase (GS) increased except for the leaf amino peptidase (As) activity. The soluble protein content, NR and GS activity of T2 (225 kg/hm^2) and T3 (300 kg/hm^2) were higher than other treatments, but decreased in the 21 days after a thesis. However, considering the cost of nitrogen fertilizer and the impact of nitrogen fertilizer on the environment, T2 was the most appropriate which could maintain high activity and stability during the entire growth period of the flag leaf and delayed the flag leaf senescence effectively.

Keywords: Amino peptidase activity, glutamine synthetase, nitrogen rate, nitrate reductase, soluble protein, winter wheat

INTRODUCTION

Wheat is an important food crop whose yield is affected by flag leaf physiological activities and the changes in enzymatic activity affect the speed of senescence of its flag leaf (Dingyi and Dang, 2007). The basic characteristics of late stage in wheat growth are that the leaf senescence and growth and development of grain carry out simultaneously. Changes in the flag leaves physiological activity have great importance to the grain growth which ultimately will affect the increase of the yield. How to extend the period of wheat leaf function to promote the leaf manufacturing more photosynthetic products, increasing grain weight and improving yield has become one of the important issues of high-yield wheat physiology. Previous studies (Mingzhu, 1996) showed that delaying the flag leaves senescence process was an important part of the high-yield wheat cultivation in the late stage of the process of the plants growth and development. The phenomenon of premature senescence of wheat flag leaf is still quite common in production due to soil and different varieties and changes in irrigation and fertilizer application measures. Thus, to study the changes in enzyme activity of wheat flag leaf after anthesis will help to elucidate the mechanism of leaf senescence and be of great significance on delaying leaf senescence process (Xusheng *et al*., 2008).

Nitrogen metabolism is one of the basic substance metabolisms in plants. Therefore, it has positive significance to study the physiological activities of flag leaf after anthesis by studying the wheat flag leaf soluble protein, activity of nitrate reductase and glutamine synthetase. The senescence physiology had been studied with the same amount of nitrogen fertilizer at different growth stages of wheat (Zhenwen *et al*., 1997; Lina *et al*., 1999); the photosynthetic characteristics of wheat under humid and drought conditions had been studied (Zhou, 1997). There is still a lack of in-depth research about the different amount of nitrogen fertilizer on wheat flag leaves regulation mechanism. The experiment under the conditions of different application amounts of nitrogen fertilizer, studied the effects of nitrogen fertilizer on activities of the enzyme of wheat flag leaf after anthesis.

MATERIALS AND METHODS

With Jimai 20 as materials, this experiment was carried out in the field in the experimental base of Qingdao Agricultural University (N36.32° E120.39°) in 2011-2012 for sandy loamy soil, the experiment set of 4 different treatments, followed by Control (CK), Treatment 1 (T1), Treatment 2 (T2), Treatment 3 (T3) and per hectare pure nitrogen of 0, 150, 225, 300 kg with urea as nitrogen fertilizer respectively. All treatments were fertilized of organic manure 3000 kg/ha, P_2O_5 225 kg/ha, K_2O 150 kg/ha. All fertilizers were applied into the field as basal fertilizer. There were 3 replicates for each treatment, a total of 12 plots and each plot area 120 m^2. Randomized block design with a population density of 1.8 million/hm^2. During 0-

Corresponding Author: Yan Shi, Dryland Technology Key Laboratory of Shandong Province in Qingdao Agricultural University, Qingdao, Shandong, China, 266109

20 cm soil, there was organic matter content of 1.16%, available nitrogen 95.3 mg/kg, available phosphorus 28.6 mg/kg, available potassium 106.2 mg/kg.

Items and methods: Soluble protein content: Using the method of Coomassie blue colorimetric (Shuji, 1994) to measure.

Nitrate reductase activity: using the methods (East Normal University of China, 1990) to measure and the colorimetry units was $NO2^{-}\mu g \cdot g^{-1}FW \bullet h^{-1}$

As: using the methods (Deborah and Storey, 1981; Gao *et al*., 2007) to measure the As, The enzyme activity units was ΔA523 • mg -1 pro • h^{-1}

GS: using the methods (Miflin and Lea, 1980; Bing *et al*., 2008) to measure the GS.

RESULTS AND ANALYSIS

Effect of different treatments on soluble protein content in wheat flag leaf: From Fig. 1, there was a high content of soluble protein at the beginning of anthesis. However, the flag leaf soluble protein content continued to decrease in 7 days after anthesis and decreased rapidly in 14 days after anthesis. In all treatments, flag leaf soluble protein content was in accordance with increasing amount of nitrogen and the sequence was T3>T2>T1>CK.

Effect of different treatments on NR in wheat flag leaf: In Fig. 2, the flag leaf NR activity decreased gradually after anthesis. The comparison among the treatments, the NR activity increased with increasing amount of nitrogen, this result indicated that nitrogen fertilization improved the leaf NR activity significantly.

Effect of different treatments on GS in wheat flag leaf: In Fig. 3, there was a high activity of GS at the beginning of anthesis, however, the flag leaf GS continued to decrease in 7 days after anthesis and decreased rapidly in 21 days after anthesis. During the treatments, the GS activity increased with increasing

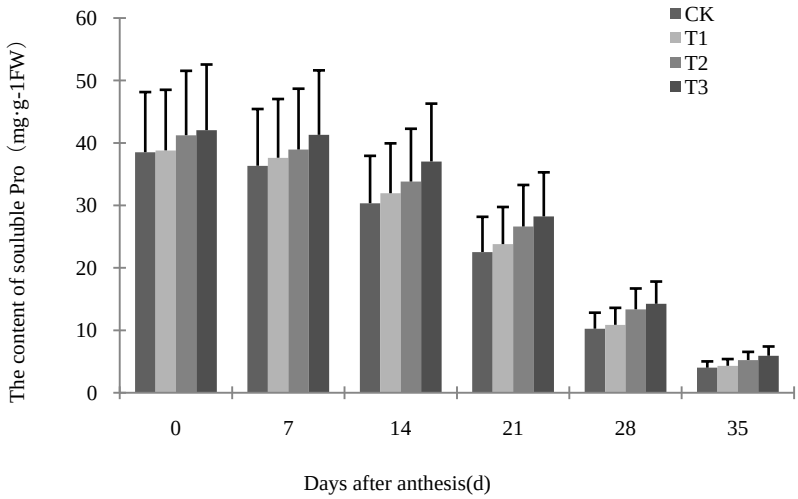

Fig. 1: Effect of different treatments on souluble protein content in wheat flag leaf

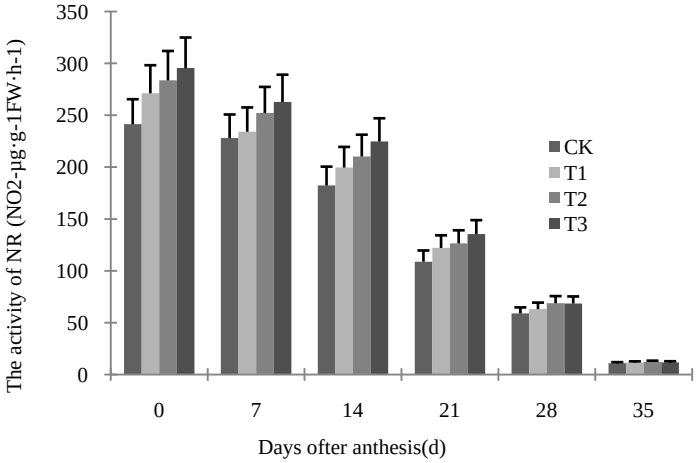

Fig. 2: Effect of different treatments on NR in wheat flag leaf

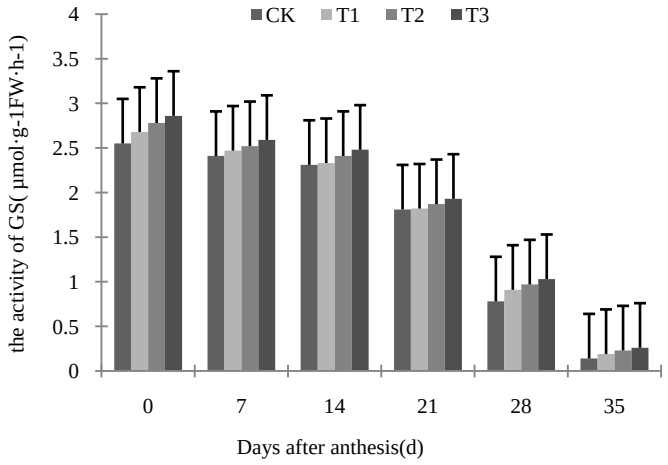

Fig. 3: Effect of different treatments on GS in wheat flag leaf

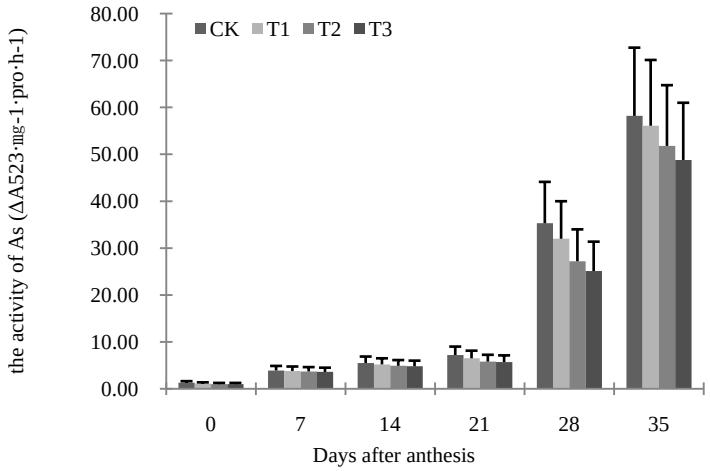

Fig. 4: Effect of different treatments on as in wheat flag leaf

amount of nitrogen, the results showed that nitrogen fertilization improved the leaf GS activity significantly.

Effect of different treatments on as in wheat flag leaf: From Fig. 4, the As gradually improved after an thesis, but the enzyme activity increased more slowly in early 21 days after an thesis and increased rapidly 21 days after an thesis. Comparison among treatments, nitrogen rate amino peptidase activity reduced the flag leaf as during each period of increasing, but in early 21 days after a thesis less difference among treatments, after differences become larger. It showed that the increasing nitrogen rate delayed the degradation of protein.

DISCUSSION AND CONCLUSION

Changes in the content of soluble substances could reflect the ability of the plant osmotic adjustment. The results of previous studies (Minjun *et al*., 2002) showed that during growth and development of wheat, the level of soluble protein content not only reflected the level of plant nitrogen metabolism, but also was regarded as an important indicator of the degree of leaf senescence, especially in wheat grain filling stage, increasing soluble protein content of flag leaf was conducive to the maintenance of the flag leaf growth and extending the photosynthetic function, so as to lay the substances basis for the accumulation of grain carbonitrides. Flag leaf soluble protein was one of the substances produced by "source" which plays an important role in increasing the libraries. Results of this study showed that the flag leaf soluble protein content was very high in initial stage of anthesis, but in 7 days after anthesis the flag leaf soluble protein content sustained to decline and decreased rapidly after 14 days after an thesis. Between each treatment, the flag leaf soluble protein content all maintained a high level with increasing amount of

nitrogen fertilize which indicated that the increase in nitrogen fertilizer is conducive to delaying flag leaf senescence.

Nitrate reductase is a key enzyme to reduce nitrate nitrogen absorbed in wheat plant into ammonia nitrogen and ammonia assimilation by pathway of GS/glutamate synthase while GS is a multifunctional enzyme in the center of nitrogen metabolism involving in a variety of nitrogen metabolism regulation (Ma and Jiguo, 1996). Improvement of the GS activity could also contribute to the enhancement of operation of nitrogen metabolism to promote the synthesis and conversion of amino acids. Previous studies have proved that (Li *et al*., 1998) nitrate reductase activity changed with different amount of nitrogen fertilizer, the overall performance was that the nitrate reductase activity maintained high level with the improvement of nitrogen fertilizer level.

Aminopeptidase plays a certain role in protein degradation of flag leaf senescence and the proteolytic enzyme activity in vivo of wheat is closely related to the degradation of the protein and in the process of protein hydrolysis of amino acids, aminopeptidase plays a major role to hydrolyze the protein completely into amino acids which will be transported to grain after anthesis through a variety of pathways for the synthesis of new proteins. The majority of studies have proved that aminopeptidase activity gradually increased with the flag leaf senescence (Gao *et al*., 2007). This experimental showed that aminopeptidase activity increased after anthesis, but in the early 21 days after anthesis the enzyme activity increased more slowly with no significant changes and after 21 days after a thesis it increased rapidly with significant changes. With increasing nitrogen fertilizer, amino peptidase activity of the flag leaves maintained a lower level which showed that increasing nitrogen fertilizer delayed the degradation of protein and was conducive to delaying flag leaf senescence.

Under the conditions of this experiment, with the increasing nitrogen fertilizer it was conducive to maintain activity of winter wheat flag leaf enzymes after anthesis, thus delaying the flag leaf senescence. But considering the input costs of nitrogen fertilizer, comparing T2 and T3, we thought T2 (225 kg/hm^2) was more appropriate for the nitrogen application.

ACKNOWLEDGMENT

Supported by the program of “The research on the efficient use technology and demonstration of nitrogen fertilizer in main crops (201203079)”; “Integrated research and demonstration of the balanced increase in winter wheat and summer maize in Shandong Province (2012BAD04B05)”; the innovation team in wheat of Shandong province “cultivation and soil fertilizer (621135)” and The Taishan Mountain Scholar Constructive Engineering Foundation of Shandong Province.

REFERENCES

Bing, W., Y. Liang and W. Shi, 2008. Effect of nitrogen quantity on glutamine synthetase activity in gr ain and yield of spr ing maize. J. Northeast Agricultural Univ., 39(6): 14-16.

Deborah, S. and R. Storey, 1981. Proteoytic and trypsin inhibit or activity in germinating jojoba seeds (*Simmondsia chinensis*). Plant Physiol., 68: 1339-1344.

Dingyi, Z. and J. Dang, 2007. Regulative effect of nitrogen fertilization on grain yield, quality and photosynthesis of flag leaves in different wheat varieties. Plant Nutrition Fertilizer Sci., 13(4): 535-542.

East Normal University of China, 1990. Plant Physiology Experiments the Guidance [M]. People's Education Press, Beijing.

Gao, L., Y. Mao-Bing, X. Lang-Lai and S. Wen-Biao, 2007. Factors at fecting on the Rise of Aminopeptidases Activity in Wheat Leaves during Aging. Acta Agric. Boreali-occidentalis Sinica, 16(5): 72-74.

Li, C., W. Guoliang, D. Ximei, S. Xiaoling and W. Yong, 1998. On the nitrate reductase activity dynamic change of superhigh yield wheat and the relationship between itand nitrogen operation. J. Henan Vocation-Technical Teachers College, 26(2): 5-9.

Lina, J., L. Cuhnxi and D. Ximei, 1999. Effect of different nitrogen application on the physiological activity of flag leaf at the later stage of wheat. J. Shenyang Agric. Univ., 30(6): 609-612.

Ma, F. and G. Jiguo, 1996. Studies on nitrate reductase activity as indices of nitrogen nutrition diagnosis and sugar yield prediction in sugar beet. Scientia Agricutura Sinica, 29(5): 16-21.

Miflin, B.J. and P.J. Lea, 1980. Ammonia Assimilation [M]. In: Miflin, B.J. (Ed.), the Biochemistry of Plants. Academic Press, New York, pp: 169-202.

Mingzhu, S., 1996. Preliminary Report of the corn leaf nitrate reductase. Dyn. Shaanxi Agric. Sci., 1: 25-26.

Minjun, X., L. Guiru, Y. Xueju and W. Lijun, 2002. The influence of water stress to protein metabolism of winter wheat cultivars with different drought resistance ability. Agric. Res. Arid Areas, 20(3): 85-88.

Shuji, W., 1994. The Basis of Biochemical Experiments Guidance. Shanxi Science and Technology Press, Beijing, pp: 53-190.

Xusheng, S., Q. Lin and L. Li, 2008. Effects of nitrogen supply on photosynthetic characteristics at later developing stages and yield in superhigh- yield winter wheat. Plant Nutrition Fertilizer Sci., l(5): 84-84.

Zhenwen, Y., Y. Songlie and Y. Xu, 1997. Effects of nitrogen application at different growth stages on the senescence of flag leaves and grain yield in winter wheat. Scientia Agricultura Sinica, 30(2): 42-46.

Zhou, P.S., 1997. Regulationof nitrogen nutrition on photosynthetic characteristics of winter wheat on dryland. Plant Nutrition Fertilizer Sci., 3(2): 105-109.