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Full Length Research Paper

Lipid peroxidation, antioxidant enzyme activity and osmotic adjustment changes in husk leaves of maize in black soils region of Northeast China

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Two varieties of maize (*Zea mays* L.) grown in fields in Black soils of Northeast China were tested to study the variation of membrane lipid peroxidation, osmotic adjustment, and antioxidant enzyme activity in husk leaves of maize by the methods of UV spectrophotometry. The results showed that MDA content and membrane relative permeability increased continuously along with the growth of maize, and antioxidant enzyme activity in husk leaves of maize increased first and then decreased with the growing of maize, and reached peak value at grain filling period. It indicated that the decrease of antioxidant enzyme activity was closely correlated to the accumulation of MDA. Patterns of soluble sugar and proline were consistent with antioxidant enzyme activity, showing that osmotic adjustment could protect many enzymes important for cell metabolism through accumulation of soluble sugar and proline. Proline content of husk leaves was more variable than soluble sugar, which showed that proline was the predominant osmotic adjustment substance in husk leaves of maize.

Key words: Maize, husk leaves, antioxidant enzyme, membrane lipid peroxidation, total soluble sugar, proline.

INTRODUCTION

Plant senescence was a highly complicated procedure at cell, tissue and organ level, reactive oxygen species (ROS) were generated as natural products of plant cellular photosynthetic and aerobic metabolisms during the senescence of plant (Silva et al., 2010). Overproduction of ROS in plant cells could damage cellular components, including, proteins and membrane lipids (Mittler, 2002). In order to limit oxidative damage under stress condition plants developed a series of detoxification systems that breakdown the highly toxic ROS (Liu and Huang, 2000; Almeselmani et al., 2006). Plants have evolved efficient antioxidant systems that could protect them from the damaging effects of oxidative stress (Asada, 1999). These mechanisms employed ROS-scavenging enzymes, such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) (Morsya et al., 2003).

Maize (*Zea mays* L.) was one of the most important crops throughout the world. Numerous studies have demonstrated that adverse conditions could induce membrane damage, increase membrane permeability and the accumulation of free radicals in maize. As a special kind of maize leaf, husk leaf had different photosynthesis tissues (Hahnen et al., 2003) and photosynthetic pathway from foliar leaves (Yakir et al., 1991). It could contribute to grain formation more efficiently than foliar leaves (Fujita et al., 2001). There were huge amount of existing information concerning on foliar leaves in maize (Ben-Nounaa et al., 2003; Riccardi et al., 2004), but no report on antioxidant enzyme activity in husk leaves of maize. In order to analyze changes of antioxidant enzyme activity and thereby to understand the relationships between antioxidant enzyme activity and osmotic adjustment of husk leaves of maize in Black Soil Region of Northeast China, two maize varieties (ZD 958 and ND 364) living under field condition were chosen in the present experiment. Three major objectives were included in the present study: (i) to study the changes of antioxidant enzyme activity of husk leaves along with the growth of maize; (ii) to analyze changes of malondialehyde (MDA) content and membrane relative permeability during the whole growth period; (iii) to make

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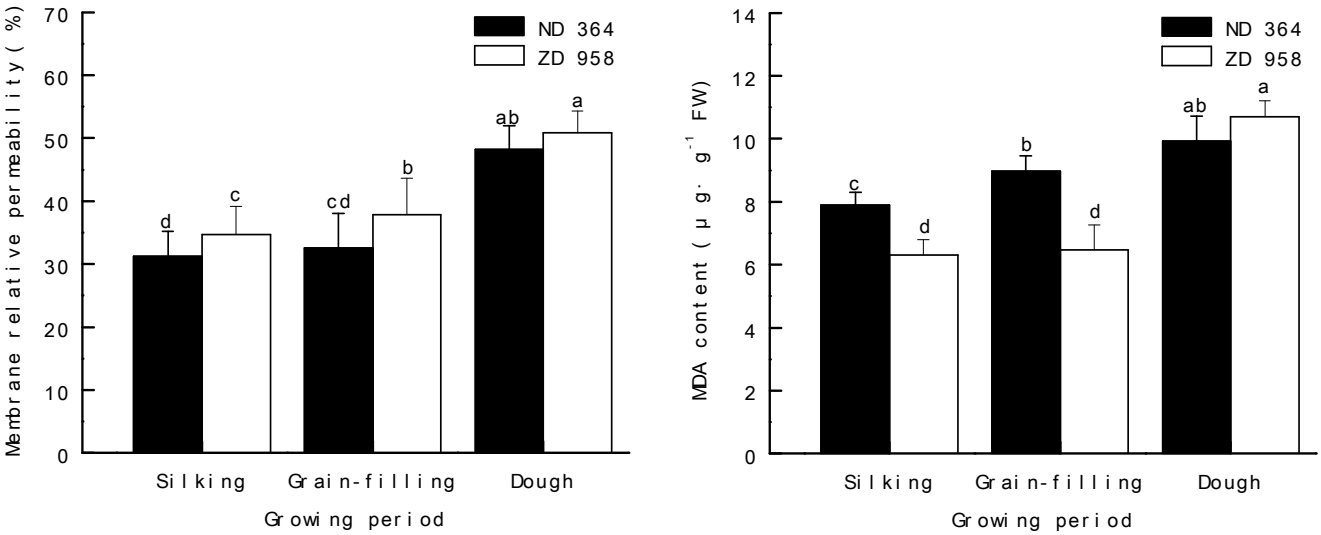

Figure 1. Membrane relative permeability and malondialehyde (MDA) content in husk leaves of maize in different growing periods. Means pairs followed by different letters are significantly different (p<0.05) by Duncan's test, n = 5.

clear relationships between antioxidant enzyme activity and osmotic adjustment of husk leaves of maize.

MATERIALS AND METHODS

Experimental design

This study was carried out at Agricultural Experimental Station (44° 12′ N, 125° 33′ E) of Northeast Institute of Geography and Agroecology. Two maize varieties, ZD 958 and ND 364 were used in this experiment. The experiment was arranged in two blocks, and each variety was cultivated in a block with an area of 1500m^2. Samples were collected in "S" shape in each block. The study was randomly conducted in five sample plots with three replicates.

Membrane relative permeability measurements

Husk leaves samples of maize were washed with deionized water, followed by the introduction of small excisions and then incubated in deionized water. Membrane relative permeability was measured using a conductivity meter (DDS-11A, China).

Protective enzyme activities determination

SOD activity was determined by measuring the photoreduction of nitroblue tetrazolium at 560 nm according to Beyer and Fridovich (1987). CAT activity was measured according to Samantary (2002). POD activity was determined using guaiacol oxidation (Bai et al., 1996). MDA was extracted with 10% trichloroacetic acid and determined at 450, 532 and 600 nm following the procedures that were described by Dhindsa et al. (1981).

Content of osmotic adjustment

Contents of soluble sugar estimations were performed according to the methods of Gao et al. (2006), and proline content was determined using the method of Zhang and Qu (2004).

Data analysis

The data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan test at 0.05 significance level to compare the means using SPSS 13.0 for Windows.

RESULTS

Membrane permeability and lipid peroxidation

Changes of membrane relative permeability and MDA content in husk leaves of two maize varieties showed the same tendencies (Figure 1). Both membrane relative permeability and MDA content were the highest at dough stage, the lowest at silking stage. Membrane relative permeability in husk leaves was lower in ND 364 than in ZD 958 during the whole growth stage. MDA content was higher in ND 364 than that in ZD 958 during the whole growth stage except at dough stage. Compared with ZD 958, the change amplitude of MDA content in ND 364 was narrow.

Osmotic adjustment

Contents of proline and TSS were measured from silking to dough period (Figure 2). The similar tendency of contents of proline and TSS was found for both varieties, that is, increased first and then decreased with the growing of maize, and finally reached the peak value at grain filling period. Both proline and TSS were higher in ND 364 than that in ZD 958 during the whole growth stage. The content of TSS had a distinctly faster increase and lower decrease in comparison with proline, and retained significantly higher value at dough stage.

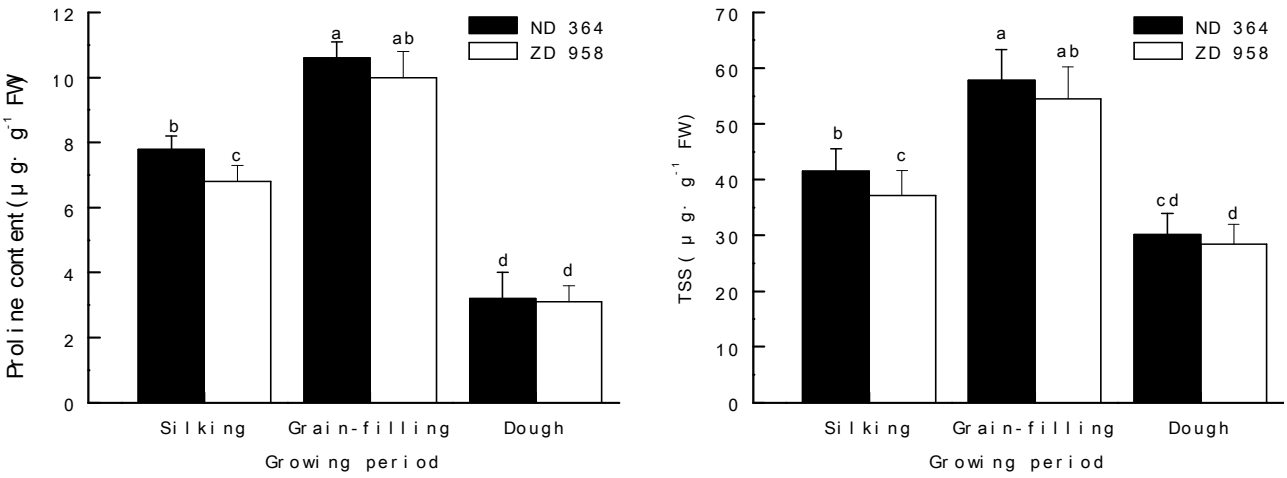

Figure 2. Content of total soluble sugar (TSS) and proline in husk leaves of maize in different growing periods. Means pairs followed by different letters are significantly different (p<0.05) by Duncan's test, n = 5.

Activities of antioxidant enzymes

Under field condition, all the antioxidant enzymes in husk leaves of maize exhibited almost the same trend with maize growing, reaching peak value at grain filling period (Figure 3). And all of them decreased from grain filling to dough period, especially CAT sharply deceased by approximately 80 percent for both maize varieties , while POD decreased smoothly by 17.39 and 28.73 percent in two maize varieties, respectively. Antioxidant enzymes activities in husk leaves of ZD 958 were significantly lower than that in ND 364.

DISCUSSION

ROS were generated as natural products during the senescence of maize, the accumulation of ROS could break the balance between ROS production and the capacity of plants to scavenge for them (Zhang et al., 2006; Herbinger et al., 2002). Increases in ROS were demonstrated by the increases in membrane relative permeability and MDA levels (Kuo and Ching, 2003). Therefore, MDA content and membrane relative permeability in the husk leaves of maize plants was measured in my study. The continuous increases of MDA content and membrane relative permeability along with the growing of maize obtained in this study was in agreement with the previous results (Ge et al., 2006). It indicated that enhancement of membrane lipid peroxidation was accompanied by exosmosis of electrolytes, an increase in membrane relative permeability, and the cell membrane integrity was injured finally. From silking to grain filling period, membrane relative permeability increases slightly in two varieties, which suggested cell membrane was not damaged severely. The extent of disorganization of cell membrane was enhanced at dough period, meanwhile, both membrane relative permeability and MDA content was lower in ND 364 than that in ZD 958, indicating the higher physiological activation in the husk leaves of former. This is important physiological basis of high production in maize.

To protect organelles from damage caused by accumulated ROS, cells express antioxidant enzymes (Foyer and Noctor, 2000). SOD, CAT, and POD are the important antioxidant enzymes because they can efficiently prevent the accumulation of O_2^- and H_2O_2 and minimize the deleterious effects of ROS (Jaleel et al., 2009). Present studies found the trend of antioxidant enzymes activity is just opposite to that of MDA content after grain filling period, it indicated that the decrease of the activities of the protective enzymes were closely correlated to the accumulation of MDA. On one hand, reduction of enzymes activities resulted accumulating in free radicals and even exceeded the injury threshold, thereby increasing MDA content and plasma membrane through direct and indirect initiation of lipid peroxidation. On the other hand, accumulation of MDA inhibited the activities of the enzymes, thus the injury to membrane was heavier (Tang et al., 2010; Ge et al., 2006). After grain filling period, both SOD and CAT activities sharply deceased , while POD activity decreased smoothly and kept at higher level in two maize varieties, suggesting that POD was the key enzyme involved in scavenging active oxygen at this time. Activity of protective enzymes in ND 364 was higher than that in ZD 958, suggesting the stronger scavenging system of active oxygen. This was helpful to keeping biological activity of husk laves and prolonging the period of photosynthetic functions. The inconsistencies of changes of antioxidant enzymes suggested that complex internal connection and cooperative action might occur in the antioxidant enzyme system of husk leaves.

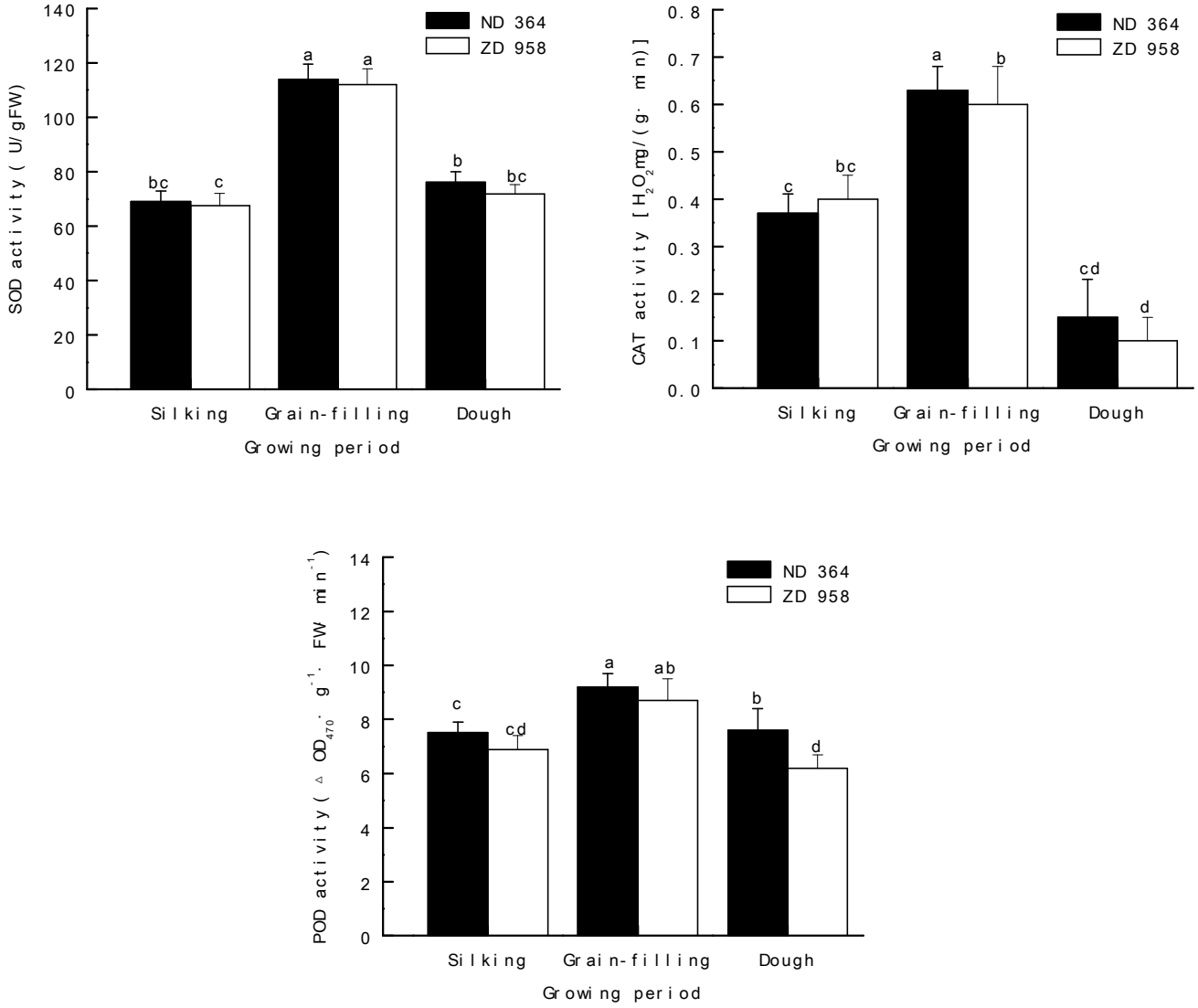

Figure 3. Superoxide dismutase (SOD) activity, catalase (CAT) activity, and peroxidase (POD) activity in husk leaves of maize in different growing periods. Means pairs followed by different letters are significantly different (p<0.05) by Duncan's test, n = 5.

Plant regulated osmotic potential through the accumulation of osmotic adjustment substance, both soluble sugar and proline were important osmotic adjustment substances in plant (Sharma and Dietz, 2006; Kishor et al., 2005). Proline could regulate cell membrane integrity by improving the hydration between protein molecules, and protect enzymes from injury through some degree of passivation (Liu et al., 2006). This was a very effective self-protecting mechanism. Soluble sugar was source of energy for synthesizing organic solutes, and it could protect enzymes with the increase of inorganic ions concentration (Zhang and Fan, 2002). Patterns of soluble sugar and proline were consistent with antioxidant enzymes, showing that osmotic adjustment could protect many enzymes important for cell metabolism through accumulation of soluble sugar and proline. And this was advantageous to the process of grain filling in maize. Proline content of the husk leaves was more variable than soluble sugar, which showed that proline was the predominant osmotic regulation substances in husk leaves of maize for regulation of osmotic potential, protecting cell from injury, and maintaining original biological processes.

In conclusion, our results suggested that osmotic adjustment could protect many enzymes important for cell metabolism through accumulation of soluble sugar and proline, and proline was the predominant osmotic regulation substances in the husk leaves of maize. The inconsistencies of changes of antioxidant enzymes activities occurred in the husk leaves of maize, suggesting that complex internal connection and cooperative action might occur in the antioxidant enzyme system

of husk leaves. Thus, it is necessary to conduct further studies on complex physiological and biochemical mechanism in order to understand the role of antioxidant enzyme in metabolism of maize.

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