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Effects of plow pan on SPAD value and chloroplast ultrastructure in leaves of spring maize

Efectos de la compactación del suelo producida por la labranza en el valor de SPAD y la estructura del cloroplasto en hojas de maíz de primavera

Li G, FT Yang, XL Jiang, FX Chen, TH Cao, LC Wang

Abstract. The aim of this study was to evaluate the impact of the plow pan on the production of spring corn. We evaluated SPAD values and chloroplast ultrastructure in premature ear leaves of spring maize at the grain-filling stage. The maize plants were grown in simulated plow pan or simulated subsoiling treatments. Plants in the simulated plow pan treatment showed irreversible damage to chloroplasts, including changes in chloroplast shape, disintegration and rupture of chloroplast membranes, and blurriness and cloudiness of grana lamellae. The chlorophyll content decreased, which is a typical characteristic of senescence. Subsoiling cultivation practices resulted in chloroplasts with a more stable ultrastructure in maize leaves. This extended the functional period of leaves after flowering, avoiding premature senescence of spring maize and increasing yields.

Keywords: Plow pan; Chloroplast; Spring corn; Premature senescence; Ultrastructure.

Resumen. El objetivo de este estudio fue evaluar el efecto de la compactación del suelo debido a la labranza en la producciòn de maíz de primavera. Evaluamos los valores de SPAD y la estructura del cloroplasto en hojas prematuras de maíz de primavera en la fase fenológica de llenado de grano. Las plantas de maíz crecieron en condiciones simuladas de compactación de suelo o de rotura de dicha compactación. Las plantas que crecieron en condiciones de suelo compactado mostraron daño irreversible en la estructura de los cloroplastos, cambios en la forma de los cloroplastos, desintegración y ruptura de la membrana de los cloroplastos, y reducción de la visibilidad en la lamela de las granas. El contenido de clorofila se redujo, lo cual es una característica tìpica de la senescencia. Las prácticas de cultivo que rompieron la compactación del suelo mostraron cloroplastos con una estructura más estable en hojas de maíz. Esto prolongó el período de actividad de las hojas luego de la floración, evitando una senescencia prematura e incrementando los rendimientos en maíz de primavera.

Palabras clave: Compactación del suelo debido a la labranza; Cloroplasto; Maíz de primavera; Senescencia prematura; Ultraestructura.

Jilin Academy of Agricultural Sciences, Key Laboratory of Northeast Crop Ecophysiology and Farming. Ministry of Agriculture, Changchun, Jilin 130033, China
Address Correspondence to: Prof. WANG Lichun, Tel.: 86013504311691, *e-mail:* li_gang201@126.com
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INTRODUCTION

Leaf senescence is a critical developmental stage for plants. Green leaves accumulate nutrients during the growth process, and the main purpose of the senescence process is to mobilize and recycle these nutrients to promote seed development and to prepare for the next generation (Diaz et al., 2005). Leaf senescence is characterized by a decrease in chlorophyll content, observed as leaf yellowing, which affects photosynthesis and nutrient uptake and accumulation. Senescence can be influenced by external factors (e.g., water, disease, injury, nutrients) and internal factors (e.g., genes, hormones, enzymes). However, even under normal conditions, premature senescence occurs in leaves of some plants. Premature senescence is not natural, but a cessation of photosynthesis during the effective growth period; therefore, it affects the normal growth and development of plants (Hao et al., 2011). The chloroplast can convert light energy into chemical energy via its membrane system, and the ultrastructure and integrity of that system are directly related to total chlorophyll content and photosynthetic efficiency (Anderson et al., 1973). Chloroplasts differ greatly among different plant species, but are relatively uniform in the same tissue. The chloroplast is also an unstable organelle, and it can change adaptively with environmental conditions (Deng & Zhou, 2006). Soil water stress has serious effects on the ultrastructure of mesophyll cells, and especially on the chloroplasts and thylakoids (Wang et al., 2008). In corn, reproductive growth after the silking and flowering stages is the critical period for yield, and chlorophyll content and intact chloroplast structure are key factors for accumulation of dry matter and high yields (Yu et al., 2010). Therefore, chlorophyll content and the integrity of chloroplast ultrastructure in leaves of maize is important for prolonging the period in which leaves remain functional after flowering.

The SPAD-502 chlorophyll meter is an instrument that transmits red light (peak wavelength 650 nm) and near-infrared light (940 nm) from light-emitting diodes. The emitted light travels through the leaf and arrives at the receiver, where the transmitted light is converted into electrical signals, amplified through an amplifier, converted into a digital signal by an A/D converter, and then calculated into a SPAD (specialty products agricultural division) value via a microprocessor (Zhang et al., 2009). Thus, SPAD values quantify the chlorophyll content of plants (Yang et al., 2009) and the SPAD-502 chlorophyll meter can be used to determine leaf chlorophyll content non-destructively (Wang et al., 2009).

The 'plow pan' is a solid layer below soil tilth formed as a result of many years of farming operations. At present, shallow topsoil and a thick plow pan are two widespread problems in the spring corn-producing areas in Northeast China and the Huanghuaihai summer maize-producing areas in China. These factors restrict corn root development and corn production, and late premature senescence has become a bottleneck for further increases in corn production (Chen et al., 2011). In this study, we grew maize plants in simulated plow pan and simulated subsoiling treatments. We evaluated their SPAD values and changes in chloroplast ultrastructure in premature ear leaves at the grain-filling stage. These data will provide a theoretical basis to solve the problem of premature senescence of spring corn by reducing or breaking the plow pan.

MATERIALS AND METHODS

The maize variety used in these experiments was "Zhengdan 958", which have high and stable yield and is the most common variety used by farmers in the region, even in China.

The study was conducted at the experimental field of Jilin Agricultural Academy in Changchun city of Jilin province. Corn seedlings were planted in PVC pipes (25 cm diameter, 75 cm long). The pipes were buried underground with the mouth of the pipe 5 cm above the ground. For the simulated plow pan treatment, the plow pan was 15 cm beneath the topsoil; it was 15 cm thick and its weight was 1.5 g/cm^3. For the simulated subsoiling treatment, the soil weight was 1.2 g/cm^3. The types of soil were light loam, and it was the same texture in both study treatments. Both treatments were each replicated in 24 tubes. The spacing between lines of tubes was 60 cm and the spacing between plants was 33 cm. Field management and fertilization were carried out according to farmers' practices [basal fertilizer was applied 500 kg/ha compound Fertilizer ($N:P_2O_5:K_2O$ = 15:15:15), seed fertilizer 50 kg/ha ammonium dihydrogen phosphate, top dressing 200 kg/ha $CO(NH_2)_2$ at the big trumpet period. Chlorophyll content was determined using a SPAD-502 chlorophyll meter (Minolta, Osaka, Japan) with 24 replications each treatment.

Ear leaves were sampled after 10 days of fully leaf expansion at the grain-filling period. The leaves were cut into 2–3 mm × 3–5 mm strips at a point one-third of the way along the leaf, along the central main vein on both sides. The strips were immediately immersed in 2.5% glutaraldehyde solution (in 0.1 M phosphate buffer, pH 7.2), and then kept at 4 °C. After removal from the glutaraldehyde solution, the strips were washed three times with 0.1 mol phosphate buffer (pH 7.2), and then dehydrated in an acetone series (30, 50, 70, 80, 90 and 100%) before being embedded in Spurr's epoxy resin. The resin was polymerized at 37 → 45 → 70 °C in an incubator and then cut into 50 nm slices with a LEICAUC6i-type slicer. The sections were stained with uranyl acetate and lead citrate, and then observed and photographed under a JEM-123O transmission electron microscope (Hitachi, Japan).

Data was analyzed by one-way analysis of variance using SPSS 11.5 for Windows and Excel. The data presented are the means of 24 replications.

RESULTS

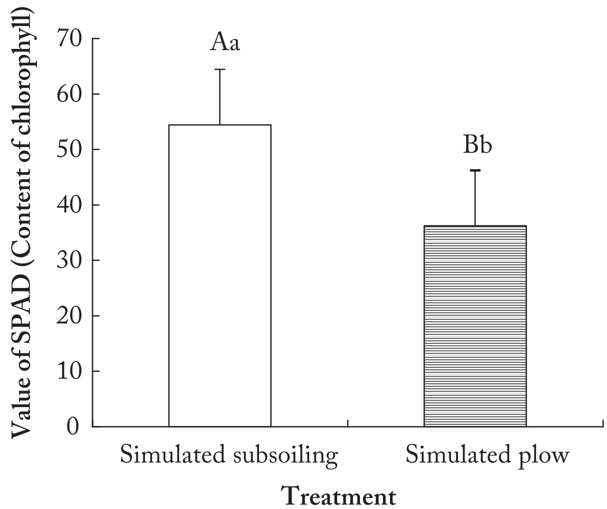
Growth and development of maize. Compared with the simulated deep plowing treatment, the growth and development of maize from the simulated plow pan treatment was lower and weaker, and maize from the simulated plow pan treatment had more premature leaves (Fig. 1).

(A) The simulated plow pan treatment, the plow pan was 15 cm beneath the topsoil; it was 15-cm thick, (B) The simulated subsoiling treatment, where no plow pan.

Fig. 1. Growth and development of maize from two treatments. (A) The simulated plow pan treatment, the plow pan was 15 cm beneath the topsoil; it was 15 cm thick, (B) The simulated subsoiling treatment, where there was no plow pan.

Fig. 1. Crecimiento y desarrollo de maíz expuesto a dos tratamientos. (A) Tratamiento de compactación de suelo simulado. La compactación del suelo estaba a 15 cm de profundidad desde la superficie del suelo; el grosor de dicha compactación era de 15 cm. (B) Tratamiento de simulación de rotura de la compactación del suelo, donde no estaba dicha compactación.

Chlorophyll contents of corn leaves. Figure 2 shows the chlorophyll contents of corn leaves from plants in the two treatments. The SPAD value for leaves in the simulated plow pan treatment was 36.2, compared with 54.4 in the simulated deep plowing treatment. The difference was significant



The simulated plow pan treatment, the plow pan was 15 cm beneath the topsoil; it was 15-cm thick. The simulated subsoiling treatment, where no plow pan.

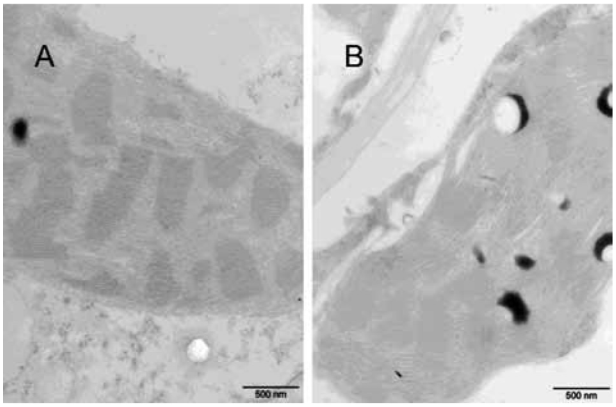
Fig. 2. Effect of different treatments on content of chlorophyll in leaf of maize. The simulated subsoiling treatment, where there was no plow pan. The simulated plow pan treatment, the plow pan was 15 cm beneath the topsoil; it was 15 cm thick.

Fig. 2. Efecto de diferentes tratamientos en el contenido de clorofila de hojas de maíz. Tratamiento de simulación de rotura de la compactación del suelo, donde no estaba dicha compactación. Tratamiento de compactación de suelo simulado. La compactación del suelo estaba a 15 cm de profundidad desde la superficie del suelo; el grosor de dicha compactación era de 15 cm.

($p<0.05$), and was also visible to the naked eye in terms of leaf shape and color. The data showed that subsoiling or breaking of the plow pan can increase the leaf chlorophyll content.

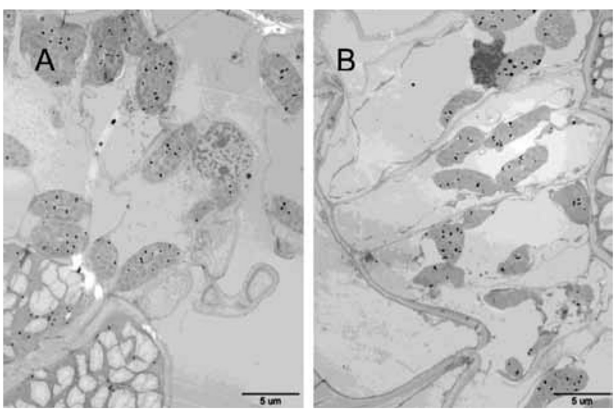
Ultrastructure of chloroplasts in corn leaves. The ultrastructure of chloroplasts in leaves of corn from the two treatments is shown in Figure 2. In the simulated plow pan treatment, the chloroplasts showed changes in shape, the chloroplast membranes were disintegrated and ruptured, there were regions of unevenness, and the grana lamellae were blurred and cloudy (Fig. 3B). In contrast, the chloroplasts in the simulated subsoiling treatment showed intact structures, normal arrangement of stroma lamellae, regions of rich grana stacking and clear layers (Fig. 3A).

The ultrastructure of cells in ear leaves of maize is shown in Figures 3 and 4. In the simulated plow pan treatment, cell walls were damaged and cell membranes were plasmolyzed. In addition, the nuclei were not intact, and chloroplasts were deformed and irregular in shape and were located away from the cell wall (Figs. 4B, 5B). In contrast, in the subsoiling treatment the cell walls were intact, the cell membranes were closely bound to the cell wall, the appearance of the cytoplasm and nucleus was normal, and chloroplasts were normal, oval-shaped, close to the cell wall, and had complete membrane systems. These characteristics indicated that the cells were healthy and functional (Figs. 4A, 5A). These data show that subsoiling treatments or breaking of the plow pan can improve plant health.



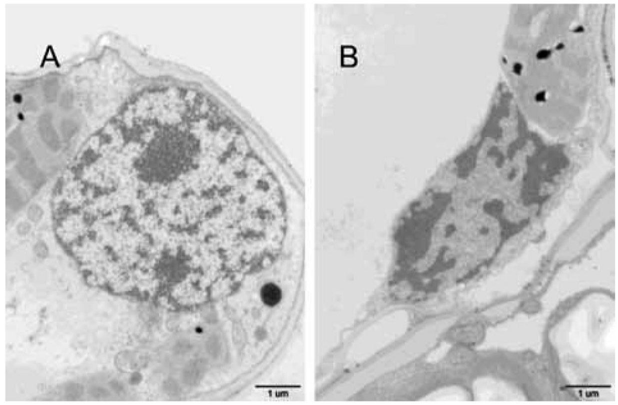
(A) The Chloroplast ultrastucture of simulated subsoiling treatment. (B) The Chloroplast ultrastucture of simulated plow.

Fig. 3. Chloroplast ultrastructure with different treatments (50000x). (A) Chloroplast ultrastucture of simulated subsoiling treatment. (B) The Chloroplast ultrastucture of simulated plow..
Fig. 3. Ultraestructura del cloroplasto con diferentes tratamientos (50000x). (A) La ultraestructura del cloroplasto en el tratamiento simulado de rotura de la compactación del suelo. (B) Ultraestructura del cloroplasto en la simulación de un suelo arado.



(A) The Chloroplast ultrastucture of simulated subsoiling treatment. (B) The Chloroplast ultrastucture of simulated plow.

Fig. 4. Leaf parenchyma cell ultrastructure with different treatments. (A) Chloroplast ultrastucture of simulated subsoiling treatment. (B) The Chloroplast ultrastucture of simulated plow.
Fig. 4. Ultraestructura de las células del parénquima foliar bajo diferentes tratamientos (5000x). (A) La ultraestructura del cloroplasto en el tratamiento simulado de rotura de la compactación del suelo. (B) Ultraestructura del cloroplasto en la simulación de un suelo arado.



(A) The Chloroplast ultrastucture of simulated subsoiling treatment. (B) The Chloroplast ultrastucture of simulated plow.

Fig. 5. Leaf parenchyma nucleus ultrastructure with different treatments (20000x). (A) Chloroplast ultrastucture of simulated subsoiling treatment. (B) The Chloroplast ultrastucture of simulated plow.
Fig. 5. Ultraestructura del núcleo de las células del parénquima foliar bajo diferentes tratamientos (20000x). (A) La ultraestructura del cloroplasto en el tratamiento simulado de rotura de la compactación del suelo. (B) Ultraestructura del cloroplasto en la simulación de un suelo arado.

DISCUSSION

In this study, the simulated plow treatment resulted in changes to chloroplast shape, disintegration and rupture of chloroplast membranes, formation of regions of unevenness and blurred and cloudy lamellae. The chloroplasts showed irreversible damage, accompanied by a decrease in chlorophyll content. These are typical characteristics of senescence.

Previous studies showed that root growth and distribution and nutrient uptake and utilization were substantially affected by soil compaction stress, and yield could be improved by decreasing soil compaction (Wang et al., 2010; Wang et al., 2011). In this study, the simulated subsoiling treatment maintained the health and functionality of the leaf even at a late stage of reproductive growth, indicating that the functional period of leaves was extended by the subsoiling treatment. These data show that subsoiling cultivation practices can result in healthier plants with chloroplasts that are more stable than those in plants grown in a plow pan. The extension of the functional period of leaves after the flowering period helps to reduce the problem of premature senescence of spring corn and increases yields.

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