



## Effects of Manganese Soaking and Gibberellins Spraying on Wheat Photosynthetic Characteristics and Yield

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### ABSTRACT

Manganese is required for wheat growth, as it is directly involved in several aspects of photosynthesis; however, manganese content varies widely in agricultural soil. An experiment was conducted to investigate the effects of varying levels of manganese in wheat, and to quantify the suitable concentration of manganese under which a high yield of wheat could be obtained. The experiment consisted of manganese seed treatments (seed soaking, with four different concentrations and seed dressing) and spraying with gibberellin (four different concentrations) during the flowering stage. During the wheat growth period, chlorophyll content, photosynthetic parameters, and output were determined. Our results revealed that seed treatment with manganese, in combination with spraying gibberellin during the flowering stage significantly enhanced the photosynthetic efficiency and chlorophyll content in wheat, and resulted in an increase of wheat grain yield by 168%. The seed treatment with manganese application only increased yield by 88.75%. Seed treatment can also affect the late growth of wheat, improve the photosynthetic parameters and chlorophyll content, and improve the wheat yield. In calcareous soil in the northwest of China, moderate application of manganese fertilizer or a moderate amount of manganese pollution is beneficial to wheat production. On the basis of the above results, wheat could be used as an alternative crop when some manganese pollution exists in soil.

### INTRODUCTION

Manganese is one of the necessary microelements for the growth of wheat, as it is directly involved in photosynthesis, photolysis of water, and transfer of electrons (Glowacka 2014, Wu et al. 2010). In addition, manganese is also an important element in superoxide SOD and plays an important role in maintaining the normal structure of chloroplast membranes (Ren & Liu 2007). The content of manganese in the soil is affected by the pH value of the soil, moisture, the quality of the soil, the content of organic matter in the soil, the aeration of the soil, and so on (Li et al. 2010). The content of manganese in different types of soil also varies greatly. For example, the manganese content in alluvial soil and in acidic soil is relatively high, while in calcareous soil and in purple sandstone soil is relatively low (Yin et al. 2016). In recent years, the content of manganese in the soil has increased because of the exploitation of manganese and the discharge of ore tailings. The excessive manganese in the soil has been shown to decrease the productivity of plants, affects the yield and quality of plants, and impacts the human

health through the food chain (Xu et al. 2011, Huang et al. 2015). Therefore, it is important to understand the most suitable soil manganese concentration for the growth and yield of wheat (Shi & Zhu 2008).

Gibberellins are a major plant growth regulating hormone. This class of hormones regulate a variety of genes and can also affect the different stages in the life history of higher plants, such as seed germination, stem elongation, the induction and development of floral organs, the formation of the seed and the fruit, and so on (Olszewski et al. 2002, Swain & Singh 2005). A suitable concentration of gibberellins is very important for plant growth. If the gibberellin content is too low, the leaves will turn dark green, the plant will remain short, and the flowering will be delayed, but if gibberellin is in excess, the plant will have large internodes and the flowering time may be earlier. Gibberellins are widely used in agriculture. Treatment with gibberellins can improve the photosynthetic quality and the growth condition of lilies tissue culture (Fleet & Sun 2005) by increasing the net photosynthetic rate and the

light saturation point, yet decreasing the light compensation point of winter Jujube during flowering (Busov et al. 2008). At present, research has been conducted on the regulation of the biological synthetic pathway as well as its regulation of the growth mechanism of the plant (Roumeliotis et al. 2013, O'Neill et al. 2010, Walton et al. 2010).

Previous studies on manganese have focused on the effect on plant growth when treated with watering, fertilizing (Kumar et al. 2009, Gyori 2007), and intercropping of different crops (Glowacka 2014, Xia et al. 2013, Glowacka 2013), as well as how to change the manganese content in the soil and seeds of plants (Cheng & Liu 2013). In addition, the effect of manganese on seed germination and the growth of the seedlings has been investigated (Hou et al. 2011). As far as we know, no research has been conducted on the effects of manganese in seed processing in regards to photosynthesis and wheat yield. Using a potting experiment and wheat, the present research explores the effect of processing seeds with manganese and the spraying of gibberellins during the blooming period on the photosynthetic parameters of the leaves, and the accumulation of chlorophyll and dry matter. The purpose of this study was to determine the most suitable concentration of manganese for use in seed processing and if the spraying of gibberellins during the blooming period would increase the wheat yield. In addition, this study aimed to provide an experimental basis and theoretical reference for environmental offices that focus on the combination of the treatment of manganese pollution and the production of wheat.

## MATERIALS AND METHODS

**Experimental materials:** The experiment was conducted in a rainproof shed at the Institute of China Arid Region at the Northwest Agriculture and Forest Science University. The soil used for the experiment was field soil (0-20 cm) taken from the Water-saving Irrigation Experiment Station at the Northwest Agriculture and Forest Science University. It was screened after 5 mm and mixed with sawdust to a proportion of 3:2, then water was added to full moisture, and the soil was left to ferment for 20 days. Finally, the soil was put into a pot. The quality of the soil was heavy soil. Its basic physical and chemical properties were: 4.5 g.kg<sup>-1</sup> organic matter, 0.7 g.kg<sup>-1</sup> total nitrogen, 3.4 mg.kg<sup>-1</sup> rapidly available phosphorus, 2.8 mg.kg<sup>-1</sup> nitrate nitrogen, 5.4 mg.kg<sup>-1</sup> ammonium nitrogen, 27.7% water-holding capacity in the field, 4.5% water content in the soil, and the experimental pot had an inner diameter of 33.5 cm and height of 39 cm. To prevent stagnant water at the bottom of the pot, a 2 cm layer of natural mishi was put at the bottom of the pot and a 2 cm layer of fine sand was added to the pot as a filtration layer above the mishi. In addition, two PVC pipes

were added that had a diameter of 1.5 cm and length of 50 cm, with five small holes on their sides for layer irrigation and maintaining aeration in the bottom soil layers. The distance of the neighbouring holes was about 6 cm, and the part of the pipe in the soil (including the bottom of the pipe) was wrapped with two layers of window screen so that the holes could not be blocked. 15 kg of air-dried soil was put in each pot with the mixture of 0.15g potassium dihydrogen phosphate as bottom fertilizer. The wheat for the experiment was the cultivar Xinong 979.

**Experimental design:** We conducted a two-factor experiment. One factor was manganese processing during sowing, and the other factor was the spraying of different concentrations of gibberellins during the blooming period of wheat.

**Seed processing:** Seeds were processed by both mixing and soaking. Mixing consisted of using 2 g of manganese chloride tetrahydrate (MnCl<sub>2</sub>·4H<sub>2</sub>O) for 100 g of seeds, adding water only to submerge the seed (D). The seeds can be seeded immediately after mixing. Soaking consisted of four different soaking processes with different MnCl<sub>2</sub>·4H<sub>2</sub>O concentrations: 0 mg/kg (S0), 30 mg/kg (S1), 60 mg/kg (S2), 180 mg/kg (S3). Each processing was repeated three times. First, wheat seeds were sterilized with 0.3% NaClO for 5 minutes, then washed fully with distilled water, and dried with filter paper. The dry seeds were then separated into different treatments and put into one of four soaking liquids with different manganese concentrations for 6 hours.

**Spraying gibberellins during the blooming period:** Four concentrations of gibberellins were sprayed on wheat during the blooming period: 0 mg/L (G0), 60 mg/L (G1), 90 mg/L (G2), and 120 mg/L (G3). Gibberellins were sprayed after 18:00h on April 18 to 19, 2014 on clear days. The contrast was sprayed only with water.

Wheat seeds treated with manganese were sown on October 25, 2013. Twenty-five large seeds were selected and sowed in each pot. During the three-leaf period, seedlings were removed, leaving 10 healthy seedlings per pot. One litre of water with dissolved 5 g urea and 3 g potassium dihydrogen phosphate was irrigated into each pot on May 15, 2014 during the elongation period.

**Testing items and method: Photosynthetic parameters:** We measured photosynthetic rate ( $P_n$ ), intercellular carbon dioxide concentration ( $C_i$ ) and stomatal conductance ( $C_d$ ) of the flag leaf on ten days after anthesis of wheat. For measurements, we used a Li-6400 portable photosynthesis system (LI-COR, USA) and conducted all measurements between 9:00h and 11:00h on a sunny morning. The LED actinic light was set at 600  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , flow velocity of 500  $\text{mmol s}^{-1}$ , external CO<sub>2</sub> concentration of about 380  $\mu\text{mol CO}_2 \text{ mol}^{-1}$ , leaf temperature between 23°C and 24°C, rela-

tive humidity (RH) of about 14.4-19.4%, and each replicated treatment was measured five times.

**The content of chlorophyll:** Taking the flag leaf every 10 days after the spraying of gibberellins, we extracted total chlorophyll content using a mixture of acetone, ethanol and water (V/V: 4.5:4.5:1) and centrifuged it at 1600 rpm for 10 min. The extraction was done in the dark with all samples kept on ice. The extraction volume was 10 mL. The absorbance of the supernatant was measured using a dual-wavelength/double beam (UV-3000) spectrophotometer. Chlorophyll *a* and *b* were measured at 663.2 and 646.8 nm, respectively. The results were analysed based on the equations of Wellburn (1994) and then normalized to leaf dry weight. The chlorophyll concentration was calculated by Lichtenthaler's formula (Lichtenthaler & Wellburn 1983).

**Dry matters:** After the harvest, the wheat samples were separated based on leaf, stem, coleoptile, grain, first heated for 2 hours at 105°C, dried to constant weight at 60°C, and then weighed.

**Yield:** In the mature period, during the maturity phase, each treatment harvested five plants, the single plant yield was taken as the average yield of these five plants; the same was repeated for three times.

**Statistical analysis:** The relevant data were calculated with Microsoft Excel 2010; variance analysis was conducted with SPSS19.0 statistic software and multiple comparisons and difference significance analysis were conducted for each treatment with LSD method; graphics were made with Sigmaplot 10.0.

## RESULTS AND ANALYSIS

### The Effect of Various Manganese Seed Treatments and Spraying of Gibberellins on the Yield of each Potted Wheat Plant

Our study found that the treatment of wheat seeds with various manganese concentrations and the spraying of gibberellins during the blooming period have significant effects on wheat yield (Fig. 1). The yield of plants from each treatment was higher than the control in all cases. The wheat yield from plants grown from treated seeds increased by 29.11%-88.75% compared with the treatment S0 (control). The highest yield of each plant in the S2 treatment was 2.282 g and this increased the yield by 88.75% compared with the S0. The increase was 16.8%-168% with the combination of seed treatment and the spraying of gibberellins. The highest wheat yield was from G2D at 2.4547 g, which is 168% higher than the control. The G2S1 and G3S3 treatments all performed well and increased by 102% and 93.5%, respectively, when compared with the control. The yield of

G2S3 was the lowest with only 0.187 times the control. The treatment and the spraying of gibberellins during the blooming period significantly improved the yield of wheat, and is affected by concentration.

### Effects of Various Manganese Treatments and Spraying of Gibberellins during Blooming Period on Photosynthetic Properties of Potted Wheat

**Effects on the photosynthetic rate:** Fig. 2 shows that the photosynthetic rate of the wheat flag leaf appears to be in a declining trend with the development of growth period (except G1D), and it falls rapidly 20 days after blooming and the trend becomes slower after 20 days.

In the gibberellin treatments, the photosynthetic rate of each seed in the three periods is remarkably higher than the control, which was treated with water. The average photosynthetic rate over the three growth periods in the four treatments, G0D, G0S1, G0S2, and G0S3, were 18.6%, 21.26%, 33.44%, 26.27%, respectively, higher than the G0S0. This shows that with the increase of the manganese concentration in the soaked seed treatment, the photosynthetic rate increased and there was depression effect on the photosynthetic rate above a certain concentration. With the increasing concentration of gibberellins, the photosynthetic rate increased 10 days after the start of flowering. The average photosynthetic rate in the three periods, G2D increased by 33.58% than G1D, and G3D is increased by 17.75% than G1D. Until the late filing period (30 days after start of flowering), the photosynthetic rate of treatment G3 was relatively concentrated and was lower among the gibberellin treatments. The difference of the three other seed treatments was relatively distinct and G1S1, G1S2, G2D, G2S1, G3S3, G0S2, and G0D all performed well.

In the treatment G0, the photosynthetic rates of each seed treatment were higher than the control. Among them, treatment G0S2 was the highest, G0S3 the second, and G0S1 and G0D were the lowest.

**Effects on stomatal conductance:** Stomatal conductance decreases first, then increases, and decreases again after the bloom and during the early filling period (Fig. 3). In the G3, G0 treatment always decreases with the stomatal conductance of the growth of wheat. Stomatal conductance in the G1D, G1S2, G2S1 and G2S2 treatments has increased in the late filling period. Stomatal conductance in the G0 treatments (except G0S3) was higher than in each G3 treatment 30 days after blooming in the final filling period. This shows that the concentration of G3 can possibly increase the stomatal conductance of wheat in the late growth period. Stomatal conductance of wheat treated with G3 10 days after blooming is higher than stomatal conductance of wheat in

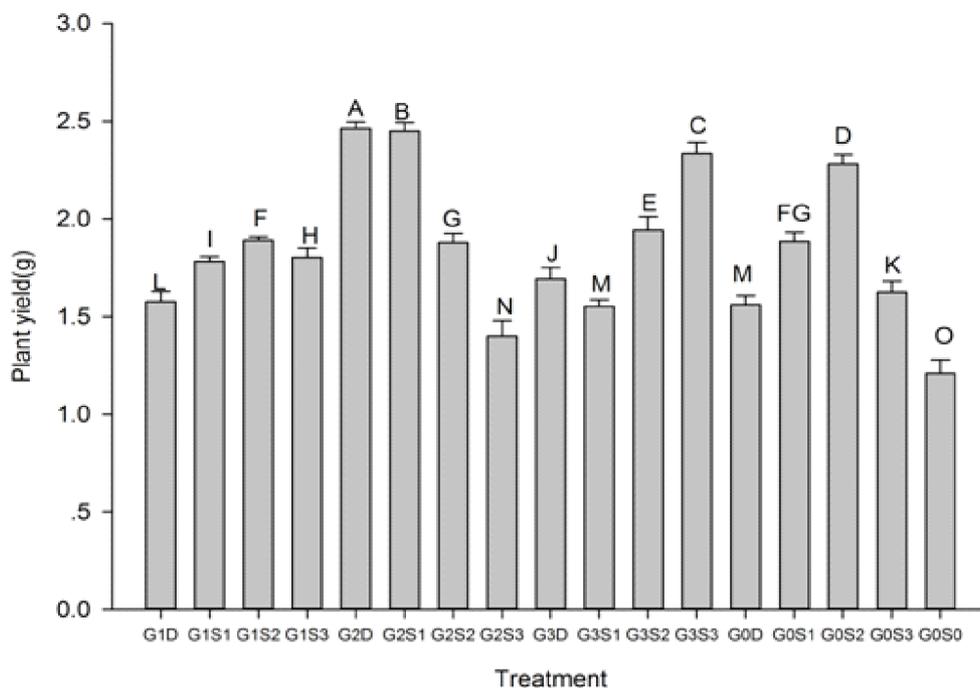


Fig. 1: Effects of manganese seed treatment and spraying of gibberellins during the blooming period on wheat yield.

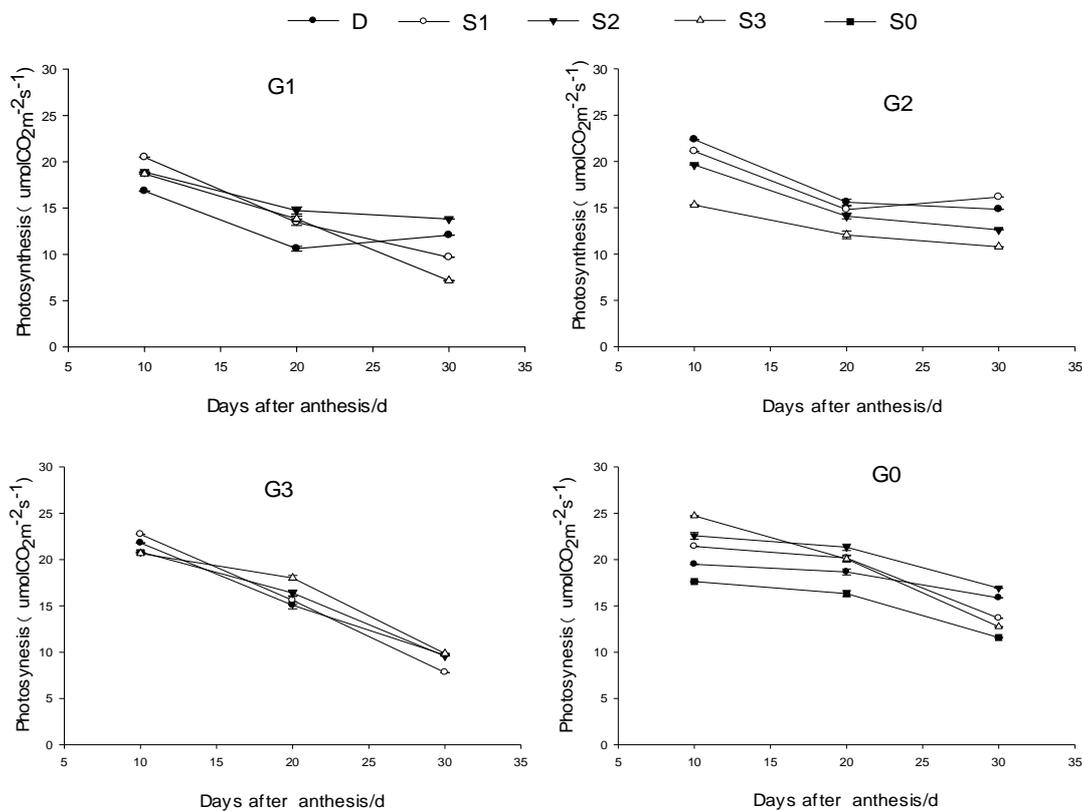


Fig. 2: Effects of manganese seed treatments and spraying of gibberellins during the blooming period on the photosynthetic rate of the flag leaf of potted wheat.

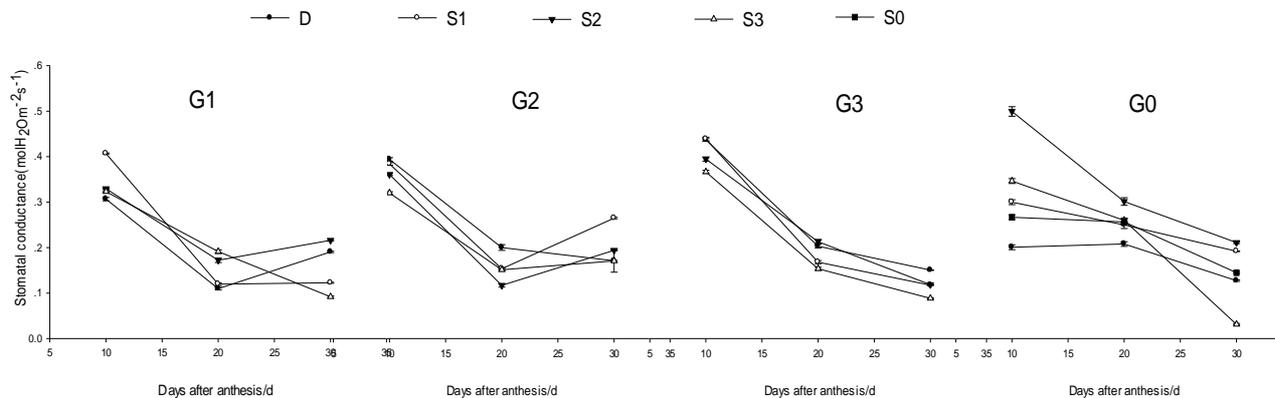


Fig. 3: Response of stomatal conductance of the wheat flag leaf to manganese seed treatments and spraying with gibberellins during the blooming period.

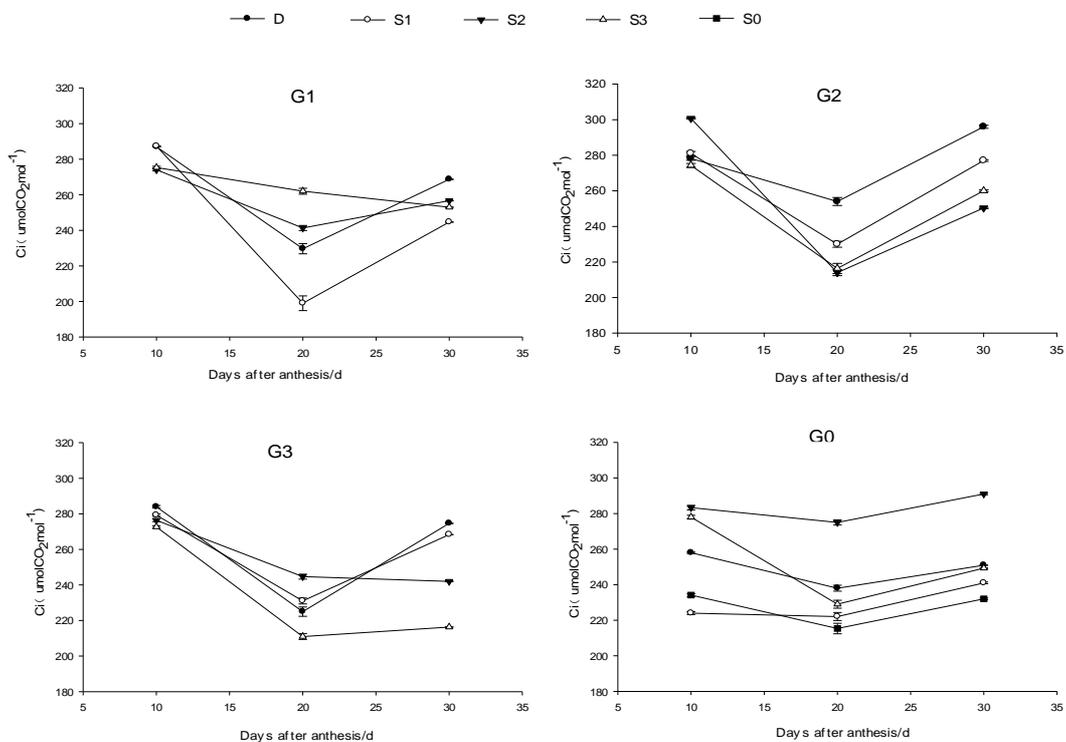


Fig. 4: Effects of manganese seed treatment and spraying of gibberellins in the blooming period of wheat.

the G1 and G2 treatments. The average stomatal conductance in the filling period (except G0D and G0S3) is higher than G0S0. Among the treatments, the stomatal conductance in G2S1 and G0S2 increased by 35% compared with the control, and G2D and G3D increased by 30% compared with G0S0. The stomatal conductance in G3S3 had no difference from the control.

**Effect on the concentration of intercellular CO<sub>2</sub>:** The concentration of intercellular CO<sub>2</sub> of the flag leaf after the

blooming period of wheat appears to follow the trend of decreasing first and then increasing (Fig. 4). The average concentration of intercellular CO<sub>2</sub> in all the three treated periods (10 days, 20 days and 30 days after the bloom) was higher than the G0S0 by 24.64%-0.9%. G0S2 had the highest concentration of intercellular CO<sub>2</sub> and it was 24.64% higher than the G0S0. G2D had the second highest and it was 21.54% higher than G0S0. G2S1 was 15.64% higher than the G0S0. G0S1 had the lowest concentration of

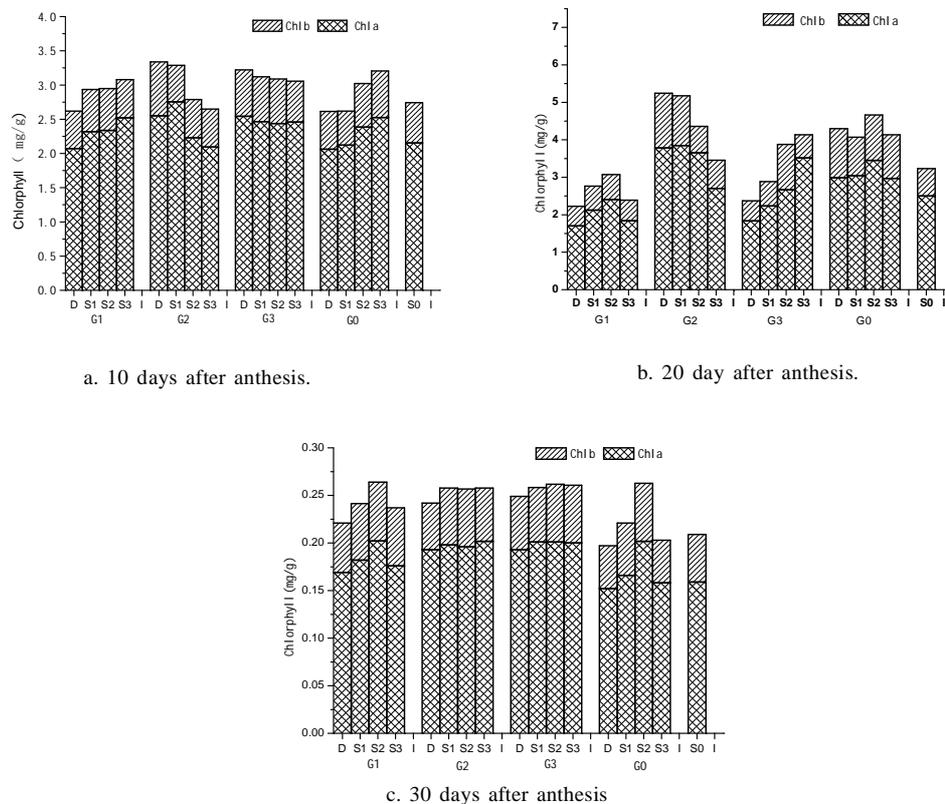


Fig. 5: Effects of different manganese seed treatments and spraying of gibberellins in the blooming period on the chlorophyll content in the flag leaf of wheat.

intercellular  $\text{CO}_2$  at 0.9% higher than the control. With the different concentrations of gibberellins, the D and S1 treatments were in correspondence with the trend of increase first and then decrease with the increase of the concentration of gibberellins. The S2 and S3 treatments had a decreasing trend with the increase of the concentration of gibberellins.

**Effects of different manganese seed treatments and spraying of gibberellins on photosynthetic pigments:** The chlorophyll content in the flag leaf of wheat increases first and then decreases with the growth period and shows a low-high-low trend (Fig. 5). The treatments with different gibberellins and manganese concentrations showed that chlorophyll *a* and chlorophyll *a+b* had a gradual increase from D to S1 and S2. During the treatment, no matter the growth period, the chlorophyll *a* content was much higher than the chlorophyll *b* content.

## DISCUSSION

**Manganese seed treatment effect on the growth of wheat in the late period and its yield:** Seed treatment and foliage spraying are two commonly used cultivation techniques in agricultural production. It is typically thought that seed

treatment only affects the growth of the seedling and has no effect until the late growth period. Therefore, seed treatment research has often focused on seed germination, the growth condition, and physical change of wheat in the seedling period (Hou et al. 2011, Gyori Z. 2007, Xia et al. 2013). This experiment shows that manganese seed treatment also has a good effect on the late growth period of wheat, and the yield increased by 88.75% compared with the G0S0.

In previous research, manganese fertilizer was sprayed on the foliage of wheat and the yield of wheat increased by 34%-77% (Yin et al. 2016) compared with untreated wheat. In research conducted by Lin (1983), manganese sulphate fertilizer was sprayed on the leaves of rice under the 4.1-5.0 pH and the yield increased by 10.8% compared with the control. Additionally, research conducted by Meng et al. (2007) indicated that when manganese fertilizer was sprayed twice in the tillering stage and heading stage, the wheat yield increased by 10.13%. The results are similar with the results from our research. Meanwhile, we can see that the manganese seed treatment has a stronger effect than the foliage spray. In addition, the manganese seed treatment compared with the G0S0, showed that the photosynthetic rate,

stomatal conductance, and the concentration of the intercellular CO<sub>2</sub> increased maximum by 33.44%, 35% and 24.64%, respectively. The chlorophyll *a* and chlorophyll *a+b* content in the manganese seed treatment was 1.27 times and 1.29 times that in the G0S0, respectively. The results of this research agree with results from previous research (Dai et al. 2009).

**The combination of gibberellins and manganese seed treatment on the yield of wheat:** Gibberellins are commonly used as growth regulators. Research on wheat mainly shows that gibberellins can regulate seed germination. The effect of gibberellin on crop yield was more studied on rice. In previous research conducted by Tian (2014), spraying gibberellins on rice in the tillering stage had a better effect than spraying during the elongation stage, with an increase of up to 9.76%. Research conducted by Cheng et al. (2011) showed that spraying rice plants with 15.0-22.5 g/hm<sup>2</sup> of gibberellins in the break period and the ear-bearing period is advantageous for the increase of thousand seed weight of rice, grain number per panicle, and maturing rate, and it can shorten the time of head sprouting, improving the yield. The present research confirmed it in wheat. In addition, the results show that a combined treatment with gibberellins and manganese seed treatment can better increase the yield of wheat, improve the photosynthetic rate of the flag leaf, improve stomatal conductance, and increase the concentration of intercellular CO<sub>2</sub>. The combination of G2D and G2S1 performs relatively better and the yield increased by 168% and 102%, respectively, compared with the control, and in the filling period, the average photosynthetic rate increased by 15.92% and 15.64%, respectively, compared with the G0S0. The stomatal conductance increased 30% and 35% compared with the control. The concentration of intercellular CO<sub>2</sub> increased by 21.54% and 15.64%. Compared with the sole manganese seed treatment, it is 1.89 times and 1.15 times higher. Thus, the manganese seed treatment together with spraying the foliage with gibberellins during the blooming period is a better way to increase the production.

**Seed treatment and gibberellins:** Although the sole manganese seed treatment and the seed treatment together with gibberellins increased production, we can still see that the concentration of S2 is better in the sole seed treatment, while S3 has some decrease, but still higher than S1 and the mixing seed, so we can conclude that S2 may not be the highest increase concentration. More experiments can be done between S2 and S3 to find out the extremely highest soaking seed concentration. Among the combinations, G2 is the best, but among the seed treatment combination are D and S1, so the concentration treated in combination should be lower compared with the sole seed treatment, or shorter during

seed treatment period. Considering environments with manganese pollution, spraying wheat with gibberellins in the blooming period can achieve an increase of wheat yield in soil with lower manganese content. If the soil manganese content is higher, gibberellins should not be sprayed in the blooming period, otherwise the yield will decrease.

## CONCLUSIONS

1. In the combined G2D, G2S1 and G3S3 treatments of manganese seed treatments and the spraying of gibberellins after blooming, the yield, the average photosynthetic rate in the filling period, stomatal conductance, and the concentration of intercellular CO<sub>2</sub> all increased remarkably compared with the control. The total chlorophyll *a* and chlorophyll *a+b* content observed in this study indicates that each combination of G2 performs better than G1 and G3.
2. The manganese seed treatment only can increase the yield of wheat and improves the photosynthetic parameter of wheat. G0S2 performs better and the yield increased by 88.75% compared with the G0S0. The photosynthetic rate, stomatal conductance, and the concentration of intercellular CO<sub>2</sub> increased by 33.44%, 35%, and 24.64%, respectively, when compared with the G0S0. The chlorophyll *a* and chlorophyll *a+b* content was 1.27 times and 1.29 times higher than the G0S0.
3. Seed treatment can affect the late growth period of wheat; moreover, it can improve the parameter of photosynthetic properties, the chlorophyll content, and finally improves wheat yield.
4. In the gibberellins treatments, the measured parameters of G2, all performed better.

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