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RESEARCH ARTICLE

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Dynamic change of organic acids secreted from wheat roots in Mn deficiency

Abstract Through solution culture experiment and liquid chromatogram technique, two wheat (*Triticum aestivum* L.) genotypes with different tolerances to Mn deficiency were used to study the dynamic change of organic acids secreted from wheat root in the conditions of no Mn, low Mn and normal Mn supply. Nine kinds of organic acids were measured in wheat root exudate. The results showed that there were significant differences of organic acids in root exudate between tolerant genotype and susceptible genotype under Mn-stressed conditions. Tolerant genotype 9023 secreted more organic acids from the plant roots than susceptible genotype CM28. The main organic acid exudate included tartaric acid, malic acid, acetic acid, maleic acid and fumaric acid. Of all these acids, the amounts of tartaric acid and malic acid in root exudate showed significant differences between the tolerant genotype and susceptible genotype under Mn-stressed conditions. The results also indicated that secreting organic acids into root rhizosphere was an active response to Mn deficiency for the tolerant genotype of wheat.

Keywords Mn deficiency, organic acids, root exudate, wheat

1 Introduction

Manganese is an essential element for plant growth. It plays an important role in many plant physiological processes, especially in photosynthesis. Mn deficiency in cereals has been found in many parts of the world, especially in calcareous soils (Reuter et al., 1988). In the early 1990s, Mn deficiency in wheat was found in a large area of Sichuan Province in China (Lu and Liu, 1994). The same problem was earlier reported in the state of Punjab in India (Nayyar et al., 1985). The deficiency usually results from low Mn availability rather than a low content in the soil, as calcareous soils often contain a large reserve of total Mn (Graham, 1988). Compared with other cereals, wheat is highly susceptible to Mn deficiency (Lucas and Knezek, 1972; Graham, 1984), but different wheat genotypes have large differences in their tolerance to Mn stress (Nayyar et al., 1985, Fang et al., 1998a).

Several hypotheses have been put forward to explain the observed differences in the tolerance of plants to Mn deficiency (Graham, 1988), but none have been established as the predominant factor in wheat. In our previous research, it was discovered that the tolerant wheat genotype showed more obvious acidification and $KMnO_4$ reduction in the root rhizosphere than the susceptible genotype (Fang et al., 2000). The acidification and Mn oxide reduction in the root rhizosphere could increase Mn bioavailability in soil. These phenomena were primarily caused by the organic acid exudate released from wheat roots.

The present experiment was conducted to demonstrate the difference in organic acid exudate between tolerant and susceptible wheat genotypes, in order to explore the tolerance mechanism of wheat to Mn deficiency.

2 Materials and methods

2.1 Genotype materials

Two wheat genotypes, 9023 (tolerant) and CM28 (susceptible) were used for the solution culture experiment. They were screened in Mn-deficient soil in previous field trials.

2.2 Solution culture experiment

The experiment was carried out in an artificial climate laboratory. Each wheat genotype had three Mn treatments:

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−Mn (no Mn supply), low Mn (Mn supply at the rate 1×10^{-8} mol·L^{-1} of Mn^{2+}), +Mn (normal Mn supply at the rate 1×10^{-6} mol·L^{-1} of Mn^{2+}); these treatments covered the range from deficiency to adequacy for wheat growth. Four replicated pots were assigned to take the ranking experiment. Mn treatments started from the fourth day after the plants were replanted in the experimental pots.

Wheat seeds were surface-sterilized with 3% H_2O_2 for 10 minutes, then washed three times with deionized water and pre-rinsed in the saturated $CaSO_4$ solution for 30 minutes. Pre-treated seeds were planted in moistened quartz sand for germination at (25 ± 2)°C. Uniform 5-day-old seedlings were selected and their endosperms were removed with tweezers to reduce the residual effect of seed Mn. The seedlings were mounted in polyvinyl chloride lids, which were placed in clean porcelain pots (1 L in volume) with 20 plants per pot. The plants were grown in deionized water for one day, then in half-strength nutrient solution for two days and from the fourth day onwards, in full-strength solution, which was replenished every three days. The plants were grown in controlled conditions with temperature at (25 ± 2)°C/(10 ± 2)°C (day/night) and a photoperiodic regime of 14-hour daylight. Photosynthetic radiation at plant tops was approximately 260 μmol·m^{-2}·s^{-1}. All equipment used to grow the plants was washed in 10% nitric acid.

The pots contained a continuously aerated solution of the following composition (unit: mol·L^{-1}): K_2SO_4, 0.75×10^{-3}; $MgSO_4 \cdot 7H_2O$, 0.65×10^{-3}; KCl, 0.1×10^{-3}; $Ca(NO_3)_2 \cdot 4H_2O$, 2×10^{-3}; KH_2PO_4, 0.25×10^{-3}; H_3BO_3, 1×10^{-6}; $ZnSO_4 \cdot 7H_2O$, 1×10^{-6}; $(NH_4)_6MoO_4 \cdot 4H_2O$, 5×10^{-9}; $CuSO_4 \cdot 5H_2O$, 1×10^{-7}; Fe-EDTA, 1×10^{-4}. The solution pH was initially adjusted to 6.2 ± 0.1 with 0.2 mol·L^{-1} KOH.

2.3 Root exudate collection

The wheat root exudate was collected on the 6th, 14th, 22nd, 38th, and 44th days after the Mn treatment. After 2 hours of exposure to light in the morning, the plants were taken from the pots and washed three times with deionized water. The plant roots were placed in a dish with 100 mL deionized water. After 2 hours of aerated culture, the plants were returned to the solution pots. At the same time, 20 mg·L^{-1} thyme camphor was added to the dish containing root exudate to inhibit the decomposition of bacteria. The exudate solution was then filtered and concentrated at 55°C until the solution dried. The exudate was re-dissolved in 10 mL deionized water and transferred to test tubes for high-pressure liquid chromatography (HPLC) analysis. The root volume was measured using the water volume method.

2.4 Analysis of organic acids in root exudate

Prior to the sample measurement, the root exudate solution was filtered with a 0.45 μm filter film. The high-pressure liquid chromatography (HPLC, Waters 996) was used to isolate organic acids in root exudate and determinate their contents. The chromatographic conditions are described by the following: Column: Hypersil ODS C_{18} (5 μm, 3.9 mm × 150 mm); Mobile phase: 0.018 mol·L^{-1} KH_2PO_4 (pH 2.2); Injection volume: 20 μL; Flow rate: 0.35 mL·min^{-1}; Column temperature: 27°C; Detector: Waters 996 PDA Spectrophotometer, UV at 214 nm. Determination of organic acids was done through the standard reagent method and the contents of these organic acids were determined by the peak area method.

3 Results

Nine kinds of organic acids were analyzed in this experiment, including tartaric acid, malic acid, acetic acid, maleic acid, fumaric acid, lactic acid, citric acid, succinic acid and oxalic acid. Five of the nine kinds of organic acids were found in the wheat root exudate, including tartaric acid, malic acid, acetic acid, maleic acid and fumaric acid.

3.1 Dynamic change of tartaric acid

9023 is a wheat genotype tolerant to Mn deficiency. Figure 1 shows that in 9023 root exudate, the amount of tartaric acid in −Mn and low Mn treatments was significantly higher than that in +Mn treatment during the growing time from 14–44 days after sowing. Some 38–44 days after sowing, the amount of tartaric acid in low Mn treatment was higher than that in −Mn treatment. On the other hand, CM28 is a wheat genotype susceptible to Mn deficiency. Figure 2 indicates that in

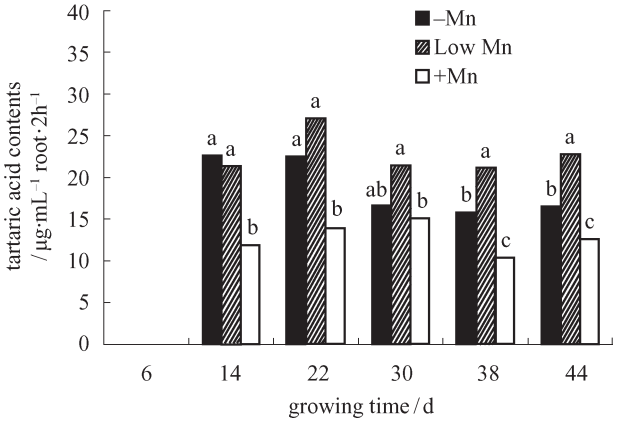

Fig. 1 Dynamic change of tartaric acid in root exudates of 9023

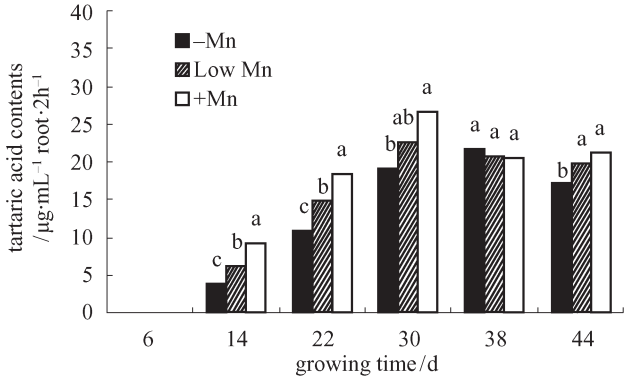

Fig. 2 Dynamic change of tartaric acid in root exudate of CM28

CM28 root exudate, the amount of tartaric acid in +Mn treatment was higher than that in −Mn and low Mn treatments on the 14th, 30th, and 44th days after sowing, without significant differences among the three treatments in the other days.

3.2 Dynamic change of malic acid

For the genotype 9023, the amount of malic acid in −Mn and low Mn treatments was significantly higher than that recorded in +Mn treatment during the entire test period, and there were no statistical differences between −Mn and low Mn treatments (Fig. 3). For the genotype CM28, there were no significant differences among the three Mn treatments for most of the growing time. On day 30 and 38, the amount of malic acid in +Mn treatment was higher than that observed in −Mn and low Mn treatments (Fig. 4).

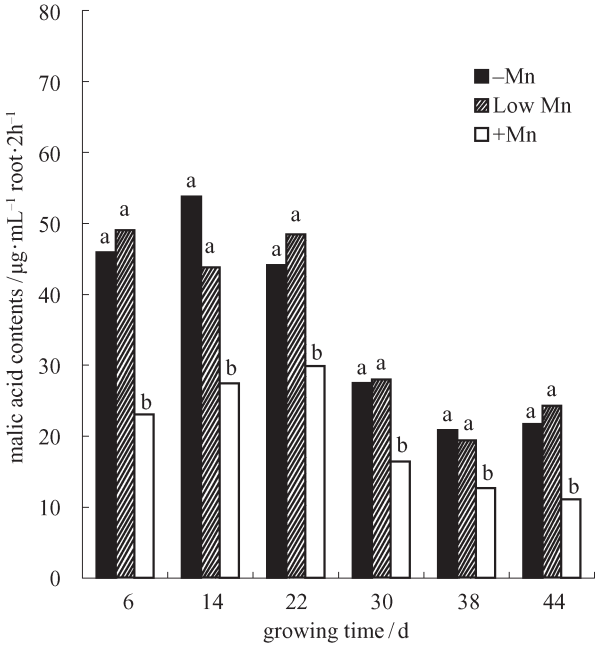

Fig. 3 Dynamic change of malic acid in root exudate of 9023

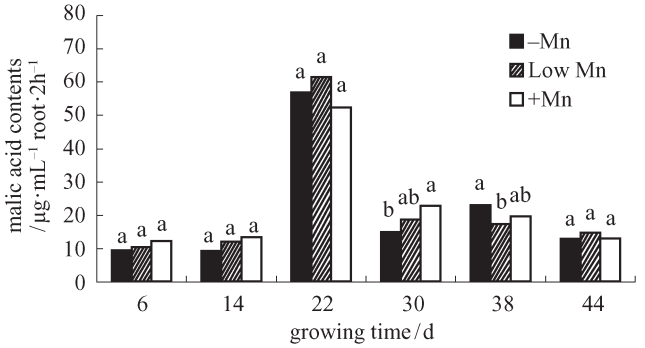

Fig. 4 Dynamic change of malic acid in root exudate of CM28

3.3 Dynamic change of acetic acid

For the genotype 9023, only on the 14th day after sowing was the amount of acetic acid in −Mn and low Mn treatments higher than that in +Mn treatment. During the other growing periods, there were no significant differences among the three treatments (Fig. 5). However, for the genotype CM28, on day 22 and 30 the amount of acetic acid in −Mn and low Mn treatments was lower than that in +Mn treatment. During the other growing periods for this genotype, there were no significant differences among the three treatments (Fig. 6).

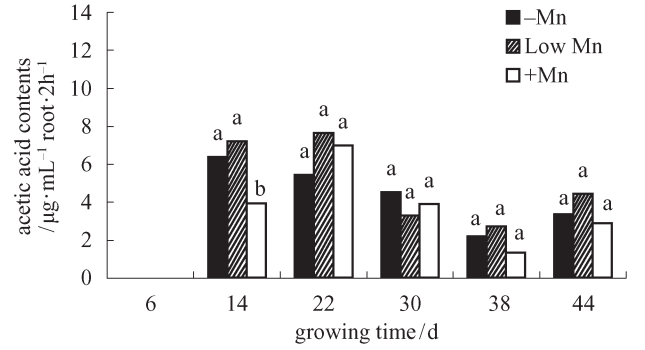

Fig. 5 Dynamic change of acetic acid in root exudates of 9023

3.4 Dynamic change of maleic acid and fumaric acid

No maleic acid and fumaric acid was detected in 9023 root exudate during the entire growing period. Compared with tartaric acid, malic acid and acetic acid, the amounts of maleic acid and fumaric acid in CM28 root exudate were extremely low, and the amounts of these two organic acids in +Mn treatment were higher than those in −Mn and low Mn treatments (Fig. 7 and Fig. 8).

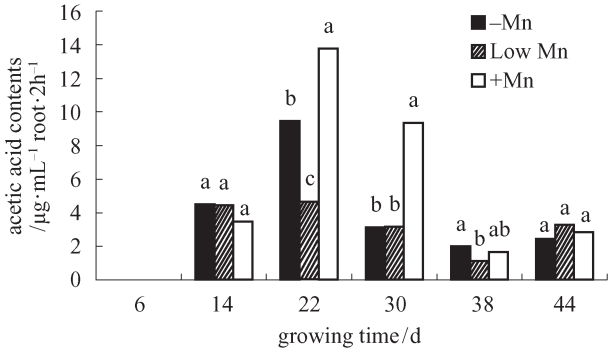

Fig. 6 Dynamic change of acetic acid in root exudates of CM28

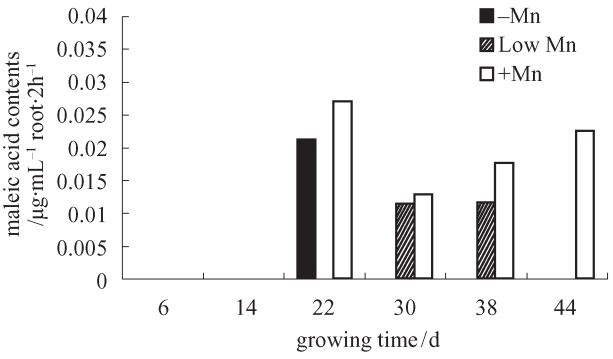

Fig. 7 Dynamic change of maleic acid in root exudate of CM28

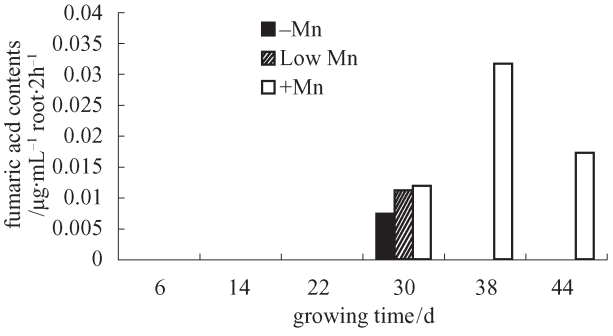

Fig. 8 Dynamic change of fumaric acid in root exudate of CM28

3.5 Change of total amount of organic acids

Table 1 shows the changes in the total amount of organic acids in the root exudate. In −Mn and low Mn treatments, the total amount of organic acids in 9023 root exudate was higher compared with that of the CM28 root exudate during the entire culture time. However, in +Mn treatment, the total amount of organic acids in 9023 root exudate was lower than that of the CM28 root exudate.

Table 1 Comparison of the total amount of organic acids in root exudate of two wheat genotypes

growing time/d	total amount of organic acids/ug·mL^{-1} root·2h^{-1}					
	−Mn		Low Mn		+Mn	
	9032	CM28	9032	CM28	9032	CM28
6	50.98	35.37	49.01	33.28	12.20	23.01
14	82.79	43.73	72.23	31.65	18.69	43.25
22	77.01	72.04	83.24	81.06	50.77	84.49
30	48.62	36.90	52.66	44.55	35.38	58.52
38	46.48	38.76	43.16	38.99	24.30	41.88
44	31.52	29.25	51.40	45.77	23.21	36.95

4 Discussion

Compared with barley, wheat is highly susceptible to Mn deficiency, but large differences between wheat genotypes still exist (Fang et al., 1998a). In our previous experiments, it was found that the differences in root volume, rooting depth and Mn requirements of the plants were not the main causes for the tolerance to Mn deficiency (Fang et al., 1998b).

There is a general agreement that Mn^{2+} is the main form for plant uptake, but in Mn-deficient soils, Mn is predominantly present as insoluble Mn oxides (eg $Mn^{II}O$, $Mn^{IV}O_2$) or carbonates which cannot be utilized by plant roots. Thus, it seems that such a mechanism of the tolerance would involve processes such as chemical reduction or rhizosphere acidification (Godo and Reisenauer, 1980; Huang et al., 1994). Our previous experiments confirmed that there did exist some differences in chemical reduction and rhizosphere acidification between the tolerant and susceptible wheat genotypes (Fang et al., 2000).

This experiment demonstrated that there were significant differences of the organic acids in root exudate between the tolerant and susceptible wheat genotypes. Under the Mn-deficient conditions (−Mn and low Mn), the tolerant genotype (9023) could release more organic acids into the rhizosphere than that of the susceptible genotype (CM28), but if there was enough Mn supply, the total amount of organic acids released from the tolerant genotype was much lower than that from the susceptible genotype. This result indicates that the response of the tolerant wheat genotype to Mn deficiency is an active reaction. Although five kinds of organic acids were found in the wheat root exudate, the main organic acids were tartaric acid, malic acid and acetic acid. The amount of the other two organic acids (maleic acid and fumaric acid) in the wheat root exudate was extremely low. Of the three organic acids in the wheat root exudate, tartaric and malic acids play important roles in the development of Mn deficiency tolerance. The active release of organic acids from tolerant wheat roots results in rhizosphere acidification and Mn reduction, which could improve Mn bioavailability to wheat plants in Mn-deficient soil.

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