

Correction of Iron Deficient Yellowing of Huangguogan by Different Application of Chelated Iron under Different Mulching Material

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Abstract. In this experiment, the *asbestos chinensis* was selected as the experimental material, and it was treated with black mulch, straw mulch, horticultural cloth mulch and river sand mulch, and compared with the control group to study the correction of iron deficiency yellowing of the leaves of Huangguogan. The results showed that the application of chelated iron under different mulching materials can significantly improve the photosynthesis index and leaf fluorescence characteristics of leaves, and correct the problem of iron deficiency yellowing of yellow fruit orange. The degree of correction of leaf iron deficiency yellowing by root-chelating iron was different under different mulching materials. In general, the correction effect of river sand mulch treatment was the most obvious, and the correction effect of black mulch was the worst. It was economical, practical, simple and convenient to correct the iron deficiency yellowing of the yellow fruit by mulching the material, and it had certain promotion value.

1 Introduction

Huangguogan was a hybrid variety of Citrus in Rutaceae family. It originated from Shimian County, Ya'an City, Sichuan Province, and was a local late ripening characteristic fruit. It has three characteristics of the flowers and fruit exist at the same time, high yield and late ripening [1]. It has become the key industry of agricultural economic development in Shimian County. In recent years, it has been found through field research that local Huangguogan appeared chlorosis at the top of the new shoots from March to April, and the leaves lost luster, shrinkage, browning and cracking of the leaf margins over time [2]. It showed that the yellow leaf disease of Huangguogan in this area was caused by iron deficiency in the tree. Therefore, improving and controlling iron-deficiency yellowing of Huangguogan was of great significance to promote the development of the local Huangguogan industry.

Mulching farmland with materials such as sand, pebbles, corn grass, leaves and animal manure, was an agricultural technique with a long history of improving soil conditions and promoting crop growth [3]. With the development of science and technology, land mulch technology has become one of the indispensable cultivation techniques in agricultural production. For Huangguogan plants with rhizosphere fertilization and mulching with different materials, there were not too many relevant studies on the corrective research of its iron-deficiency yellowing. Orchard surface mulch was

different surface mulch materials had different effects on soil fertility and water and heat conditions [4]. Therefore, through applying iron fertilizer to the roots under different mulching materials, this study explored the best mulching materials for correcting iron-deficiency yellowing and promoted them to provide effective treatment of iron-deficiency yellowing diseases in the actual production of Huangguogan reference.

2 Materials and Methods

2.1 Experimental materials

Eight-years Huangguogan with the same tree vigor, management level, yellowing degree and yellowing rate was selected as the experimental material, and the material was planted in the Huangguogan planting base in Shimian County. The experimental fertilizer was high-efficiency EDDHA chelated iron, EDDHA-Fe was 6.0% and it was suitable for all kinds of fruit trees.

2.2 Experimental design

The mulching materials were mulch, straw, horticultural cloth and river sand, and they were marked as A, B, C, and D, respectively. The blank without mulching was used as a control (CK). With a single plant as the plot, a total of 15 test materials were selected. The test treatment was shown in Table 1.

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Table 1. Treatment of rhizosphere fertilization with different mulching materials.

Treatment	Experimental method
A	black mulch
B	straw mulch
C	horticultural cloth mulch
D	river sand mulch
CK	no coverage

2.3 Experimental methods

After the fertilization treatment, the determination was carried out in mid-May, mid-September, and mid-January of the following year, and they were marked as T1, T2 and T3, respectively.

2.3.1 Leaf chlorophyll content

The relative content of leaf chlorophyll was measured with SPAD-502 chlorophyll meter.

2.3.2 Photosynthetic parameters

The net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs), intercellular CO₂ concentration (Ci) and other photosynthetic parameters of Huangguogan were measured with a portable photosynthetic instrument (Li-6400), and the instantaneous water use of leaves Efficiency (WUE) was calculated [5]. The setting parameters were: the light intensity was 1400 μmol·m⁻²·s⁻¹, the CO₂ concentration was 400 μmol·mol⁻¹, and the temperature was 25 °C.

2.3.3 Chlorophyll fluorescence characteristics

The leaf chlorophyll fluorescence parameters were measured with a portable photosynthetic instrument (Li-6400) [5]. By measuring the chlorophyll fluorescence parameters, the maximum photochemical efficiency (Fv/Fm), photochemical quenching coefficient (qP) and non-photochemical quenching coefficient (NPQ) of Photosystem II (PSII) can be calculated.

3 Results

3.1 Chlorophyll content in leaves

The chlorophyll content of leaves increased slightly first, and then decreased significantly (Table 2). The chlorophyll content of leaves under different mulching materials was significantly higher than that of CK. Among the different mulching materials, the treatment effect of river sand mulching was significantly higher than that of other mulching materials, followed by horticultural cloth mulching. There was no significant difference in chlorophyll content between the black

mulching and straw mulching treatments at the T1 and T3 periods, while the chlorophyll content under the straw mulching at the T2 period was significantly higher than that of the black mulching.

Table 2. The content of chlorophyll in leaves after applying chelated iron to roots under different mulching materials.

Treatment	Leaf chlorophyll content/ mg·g ⁻¹		
	T1	T2	T3
A	1.74±0.015c	2.04±0.017d	0.74±0.015c
B	1.76±0.012c	2.48±0.021c	0.76±0.012c
C	1.81±0.009b	2.66±0.029b	0.81±0.009b
D	1.86±0.015a	2.88±0.015a	0.86±0.015a
CK	1.25±0.019d	1.89±0.011e	0.65±0.015d

Note: Different lowercase letters within columns indicate significant differences ($P<0.05$) between different treatments. The same below.

3.2 Determination of photosynthetic parameters

3.2.1 Determination of leaf net photosynthetic rate and leaf stomatal conductance

The Pn of leaves with different treatments increased first and then decreased (Table 3). Compared with CK, different mulching materials treatments can significantly increase the Pn of leaves. The Pn of leaves under river sand mulching increased the most, and was significantly higher than that of other mulching treatments. The Pn of leaves under black mulching increased the least, and it was significantly lower than other mulching treatments.

Table 3. Net photosynthetic rate of leaves after applying chelated iron to roots under different mulching materials.

Treatment	Pn/(μmol·m ⁻² ·s ⁻¹)		
	T1	T2	T3
A	1.49±0.024d	3.96±0.019d	1.13±0.032d
B	3.17±0.026b	6.24±0.020b	2.87±0.011b
C	2.94±0.015c	5.32±0.021c	2.34±0.015c
D	3.61±0.034a	7.21±0.009a	3.19±0.012a
CK	1.05±0.023e	3.31±0.010e	1.01±0.009e

After treatment with different mulching materials, the Cs of the blades can be significantly increase, and the Cs of the blades first increased and then decreased significantly (Table 4). In the T1 and T2 periods, CK had the lowest Gs, which was significantly lower than that of the other mulching material treatments. In the T1 period, the Cs under the river sand mulch was the largest, which was significantly higher than that of other mulching treatments; while in the T2 and T3 periods, the Cs under the river sand mulch and the horticultural cloth mulch was the highest, and there were no significant differences.

Table 4. Stomatal conductance of leaves after applying chelated iron to roots under different mulching materials.

Treatment	Gs/($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)		
	T1	T2	T3
A	0.127±0.002d	0.195±0.002c	0.016±0.001b
B	0.155±0.002c	0.214±0.002b	0.023±0.001b
C	0.170±0.001b	0.235±0.002a	0.032±0.001a
D	0.176±0.001a	0.239±0.001a	0.035±0.001a
CK	0.113±0.001e	0.173±0.001d	0.019±0.003b

3.2.2 Determination of intercellular CO₂ concentration, transpiration rate and instantaneous water use efficiency in leaf

Treatments with different mulching materials can significantly increase the Ci concentration of leaves, and its concentration first increased and then decreased (Table 5). During the T1 and T3 periods, the Ci of leaves was the highest under the river sand mulching treatment, which was significantly higher than the other treatments. In the T2 period, the Ci of leaves under the straw mulching treatment was the highest.

Table 5. Leaf intercellular CO₂ concentration after applying chelated iron to roots under different mulching materials.

Treatment	Ci/($\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)		
	T1	T2	T3
A	257.00±0.11c	308.59±0.16d	221.70±0.19d
B	287.48±0.26b	334.70±0.71a	247.68±0.14c
C	251.72±0.13d	311.79±0.52c	251.72±0.13b
D	296.42±0.26a	321.47±0.08b	278.19±0.13a
CK	241.19±0.51e	293.88±0.26e	210.83±0.20e

Treatments with different mulching materials can significantly increase Tr, which first increased and then decreased significantly (Table 6). In each period, the Tr under the river sand mulching treatment was the highest, and it was significantly higher than the other treatments. The Tr increased slightly under the black mulching, which was significantly lower than the other treatments.

Table 6. Leaf transpiration rate after applying chelated iron to roots under different mulching materials.

Treatment	Tr/($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)		
	T1	T2	T3
A	1.02±0.020d	1.15±0.012d	0.57±0.003d
B	1.14±0.007c	1.24±0.007c	0.65±0.023c
C	1.26±0.009b	1.35±0.008b	0.76±0.018b
D	1.45±0.005a	1.55±0.009a	0.86±0.012a
CK	0.78±0.003e	1.07±0.015e	0.46±0.009e

Treatment of different mulching materials can significantly improve the WUE of leaves, and it first increased and then decreased (Table 7). The WUE of leaves in the straw mulching treatment was the highest, and it was significantly higher than the other mulching treatments; followed by the river sand mulching and horticultural cloth mulching treatment. In the T1 and T2 periods, the WUE of leaves in CK was the lowest. In the T3 period, the WUE of leaves under the mulching treatment and CK were 2.18 and 1.97 $\text{mmol}\cdot\text{mol}^{-1}$, respectively, and there was no significant difference.

Table 7. Leaf instantaneous water use efficiency after applying chelated iron to roots under different mulching materials.

Treatment	WUE/($\text{mmol}\cdot\text{mol}^{-1}$)		
	T1	T2	T3
A	1.47±0.007d	3.43±0.049d	2.18±0.023d
B	2.79±0.014a	5.05±0.015a	4.40±0.165a
C	2.34±0.021c	3.92±0.042c	3.10±0.090c
D	2.50±0.019b	4.64±0.032b	3.73±0.045b
CK	1.34±0.032e	3.09±0.034e	1.97±0.045d

3.3 Chlorophyll fluorescence characteristics

Treatment of different mulching materials can significantly increase the Fv/Fm of PSII (Table 8). The Fv/Fm under river sand mulching treatment was the highest, which was significantly higher than other mulching treatments. In the T1 period, the Fv/Fm under different mulching materials showed significant differences. In the T2 and T3 periods, there was no significant difference in the Fv/Fm between the straw mulching and the horticultural cloth mulching treatments, but they were both significantly higher than the black mulching treatment.

Table 8. Maximum photochemical efficiency of Photosystem II (PSII).

Treatment	Maximum photochemical efficiency (Fv/Fm)		
	T1	T2	T3
A	0.41±0.009d	0.58±0.003c	0.38±0.009c
B	0.47±0.009c	0.62±0.003b	0.43±0.012b
C	0.51±0.003b	0.63±0.012b	0.46±0.023b
D	0.58±0.008a	0.70±0.003a	0.52±0.003a
CK	0.34±0.012e	0.48±0.008d	0.27±0.003d

Treatment of different mulching materials can significantly increase the qP coefficient of PSII, which first increased and then decreased (Table 9). The qP coefficient of CK was the lowest. There were significant differences in the qP coefficient of different mulching materials. The qP coefficient under river sand mulch was the highest, followed by horticultural cloth and straw mulch, and the black mulch was the lowest.

Table 9. Photosystem II (PSII) photochemical quenching coefficient.

Treatment	Photochemical quenching coefficient (qP)		
	T1	T2	T3
A	0.42±0.008d	0.58±0.011d	0.26±0.009d
B	0.46±0.017c	0.68±0.012c	0.30±0.007c
C	0.52±0.009b	0.77±0.015b	0.34±0.015b
D	0.58±0.007a	0.84±0.015a	0.39±0.009a
CK	0.34±0.017e	0.54±0.003e	0.23±0.006e

Treatments of different mulching materials can significantly increase the PSII non-photochemical quenching coefficient (NPQ), which first increased and then decreased (Table 10). The NPQ of CK was the lowest. There were significant differences in the NPQ of different mulching materials. The NPQ under river sand mulching were the highest, followed by horticultural cloth and straw mulching, and black mulching the lowest.

Table 10. Photosystem II (PSII) non-photochemical quenching coefficient (NPQ).

Treatment	Non-photochemical quenching coefficient (NPQ)		
	T1	T2	T3
A	0.43±0.008d	0.52±0.012d	0.31±0.009d
B	0.48±0.017c	0.61±0.012c	0.35±0.007c
C	0.54±0.009b	0.70±0.015b	0.39±0.014b
D	0.60±0.007a	0.77±0.015a	0.44±0.009a
CK	0.36±0.018e	0.47±0.003e	0.28±0.015e

4 Discussion and Conclusion

The response of plant photosynthesis to PAR and Ci changes were important indicators of plant physiology and biochemistry [6]. The growth and development of green plants mainly depended on photosynthesis to fix CO₂ to synthesize organic matter. The strength of photosynthesis directly determined the level of biological and economic output. The WUE was an important indicator that coupled plant leaf photosynthesis and water physiological processes. Generally, the higher the efficiency of WUE, the stronger the ability of plants to accumulate dry matter under assimilation. The Fv/Fm was an important indicator for judging light suppression.

Some studies have shown that the chlorophyll content and leaf area of the yellowish hybrid orange leaves are significantly lower than the normal Shiranui. Leaf yellowing can significantly reduce leaf chlorophyll and photosynthetic gas exchange parameters, and also significantly reduce Fv/Fm, qP, NPQ, Pn, and photosynthesis [7]. However, these indicators can be significantly improved by applying chelated iron to the roots under different mulching materials, which

indicated that the above treatment had a certain corrective effect on the iron-deficiency yellowing of the leaves of Huangguogan, and can improve the yellowing of the leaves to a certain extent. The results of this experiment showed that the application of chelated iron to roots under different mulching materials can significantly improve leaf photosynthesis and leaf fluorescence characteristics, and correct the iron-deficiency yellowing problem of Huangguogan. Generally speaking, river sand mulching had the most obvious correction effect, followed by horticultural cloth and straw mulching, and finally black mulching had the worst correction effect. This test comprehensively evaluated some indicators related to leaf iron-deficiency yellowing, and then found a suitable method for correcting iron-deficiency yellowing. The method of river sand mulching to correct iron-deficiency yellowing was economical and practical.

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References

1. Z.H., Wang, S.F., Liu, Q.Q., Yan. (2011) *Investigation of Biological characteristics of Huangguogan in shimian county and comparison of different strains*. Northern Horticulture., **14** :20-24.
2. D.H., Wang, T.J., Li. (1991) *The selection and breeding of an excellent line of Huangguogan-Guiwankan*. China Mandarin., **20**(3): 3-5.
3. Y.Z., Liu, K.Q., Zhang, G.Q., Wang, Q.X., Li, W.R., Sun, Y., Zhai. (2005) *Research progress on dryland agricultural mulching cultivation techniques*. Chinese Agricultural Science Bulletin., **21**(5): 202-205.
4. C., Tu, J., Wang, W.Z., Liu. (2012) *Dry farmland soil respiration and its influencing factors under different mulching conditions*. Journal of Plant Nutrition and Fertilizer., **18**(5): 1107-1114.
5. M.L., Gao, Y., Qi, W.H., Song, H.R., Xu. (2016) *Effects of di-n-butyl phthalate and di (2-ethylhexyl) phthalate on the growth, photosynthesis, and chlorophyll fluorescence of wheat seedlings*. Chemosphere., **151**: 76-83.
6. Z.P., Ye. (2010) *Research progress on models of photosynthesis response to light and CO₂*. Chinese Journal of Plant Ecology., **34**(6): 727-740.
7. B., Xiong, Q.Q., Yan, G.C., Shun, X., Qiu, S., Ye, L., Liao, J.Y., Luo, L., Dai, Z.H., Wang. (2017) *Effects of Different Yellowing Degrees on the Growth and Photosynthesis of Citrus Seedlings*. Journal of Zhejiang Agriculture., **29**(9): 1506-1514.