

Effects of Cutting after Continuous Grafting on Photosynthetic Physiology of *Lycopersicon esculentum* seedlings under Cadmium Stress

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Abstract: The effects of cutting after continuous grafted on the photosynthesis physiology of *Lycopersicon esculentum* seedlings under cadmium (Cd) stress were studied by the pot experiment, and the photosynthetic pigment contents, photosynthetic characteristics and soluble sugar content of *L. esculentum* seedlings were determined. Under Cd stress, continuous grafting decreased the chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid contents of *L. esculentum* seedlings with *Cyphomandra betacea* compared with the un-grafted plants (CK). The one-grafted (G1), two-grafted (G2) and three-grafted (G3) decreased the net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs), CO₂ concentration of intercellular (Ci) of *L. esculentum* seedlings, and decreased the value of pressure deficit leaf (Vpdl) of that compared with CK. Compared with CK, the continuous grafting increased the soluble sugar contents in roots, stems, leaves and shoots of *L. esculentum* seedlings. Therefore, cutting after continuous grafted is unfavorable for the growth of the *L. esculentum* seedlings under Cd stress.

Introduction

Lycopersicon esculentum is one of the most important vegetables and cash crops in the world [1]. Grafting is commonly used in practical technology vegetable production, which can cultivate excellent varieties growing stronger, higher yield and resistance to various diseases, and it can overcome the *L. esculentum* continuous cropping obstacle and diseases [2]. At present, there are few studies on the effects of multiple grafting on plant growth, and some studies have been done on *Juglans regia* [3]. The heavy metal polluted vegetable gardens have affected the production of *L. esculentum*, especially the cadmium (Cd) [4]. The Cd is the most bio-toxic heavy metal elements, and has strong chemical activity, fluidity and toxicity in the environment, which inhibits the growth of plants, especially in high Cd concentration, and the structure of tissues was obviously deformed [5]. In this study, *L. esculentum* seedlings as scions were treated with continuous grafting on *Cyphomandra betacea*, and then cutting the shoots and planted into soil. The effects of cutting after continuous grafted on the photosynthesis physiology of *L. esculentum* seedlings were studied, and the photosynthetic pigment contents, photosynthetic characteristics and soluble sugar content of *L. esculentum* seedlings were determined, which could provide the reference for *L. esculentum* production.

Materials and Methods

Materials. The Inceptisol soil samples (purple soil in the Genetic Soil Classification of China) were collected from the Chengdu campus farm of Sichuan Agricultural University (30° 42'N, 103° 51'E) in April 2017. The basic properties of the soil were pH 6.94, organic matter 17.54 g/kg, total nitrogen 3.63 g/kg, total phosphorus 0.38 g/kg, total potassium 17.54 g/kg, alkali soluble nitrogen 195.00 mg/kg, available phosphorus 6.25 mg/kg and available potassium 191.13 mg/kg. The total Cd content was 0.103 mg/kg, and the bioavailable cadmium (Cd) content was 0.022 mg/kg [6]. The grafting *L.*

esculentum seedlings was prepared by the preliminary experiment, the seedlings were grafted in the soil bowl, and there were four treatments in the experiment, un-grafted (CK), one-grafted (G1), two-grafted (G2) and three-grafted (G3). 5 cm stems of rootstock seedlings (*C. betacea*) were selected, and 3 cm sections of *L. esculentum* grafting were used for grafting. When the grafting was completed, the soil moisture content was maintained at 80% of field capacity, and all of the seedlings were covered with transparent plastic film and a shade net. After 10 d, the transparent plastic film, the shade net and the plastic binding film were removed, and all the germinating buds of rootstocks were also removed.

Experimental Design. The soil samples were air dried and sieved to 5 mm in May 2017, then 3.0 kg of the air-dried soil was weighed into polyethylene pots (15 cm tall, 18 cm diameter), and Cd was added to the soils as $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ at 10 mg/kg [7]. The soil moisture content was maintained at 80% of field capacity for 4 weeks, and then the soil in each pot was mixed. In May, 2017, the *L. esculentum* seedlings were cutting into each pot. Each treatment was repeated three times making a total of 12 pots, and put in a completely randomized design with 10 cm spacing between pots. The soil moisture content was maintained at 80% of field capacity until the plants were harvested. Two months after planting (July 2017), the photosynthesis of each plant was determined by using LI-6400 portable photosynthesis meter (LI-COR Inc, USA). The photosynthetic parameters of the photosynthesis meters were manual control CO_2 concentration 400 $\mu\text{mol/mol}$, temperature 30 °C and light intensity 1000 $\mu\text{mol/m}^2/\text{s}$. The determination of photosynthetic parameters were net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs), CO_2 concentration of intercellular (Ci) and value of pressure deficit leaf (Vpdl), and each treatment was repeated three times. After that, the upper mature leaves of plants were collected to determine the photosynthetic pigment (chlorophyll a, chlorophyll b, total chlorophyll and carotenoid) contents [8]. 0.200 g cut into pieces of leaf samples were soaked by 20 ml mixed solution of ethanol and acetone (1:1, v/v) in the dark condition for 24 h, and then, measured the absorbencies at 663, 645, and 470 nm for chlorophyll a, chlorophyll b, and carotenoid, and calculated the chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents. Then, the whole plants were then gently removed, the roots, stems and leaves were washed with tap water followed by deionized water, and dried at 80 °C to constant weight. The soluble sugar contents of *L. esculentum* were determined by anthrone colorimetry with dry weight plant samples [8].

Statistical Analyses. Statistical analyses were conducted using SPSS 13.0 statistical software (IBM, Chicago, IL, USA). Data were analyzed by one-way analysis of variance with least significant difference (LSD) at the $p = 0.05$ confidence level.

Results and Discussion

Photosynthetic Pigment Contents in *L. esculentum* seedlings. The chlorophyll a, chlorophyll b, total chlorophyll and carotenoid contents of G1, G2 and G3 were lower than the CK. The G3 have a most decreased, the chlorophyll a, chlorophyll b, total chlorophyll and carotenoid contents of G1 decreased by 10.96% ($p > 0.05$), 13.20% ($p > 0.05$), 11.48% ($p > 0.05$) and 20.71% ($p > 0.05$), respectively, compared with CK, and the photosynthetic pigment content of G3 has a slight decreased than the CK, the chlorophyll a, chlorophyll b, total chlorophyll and carotenoid contents decreased by 2.34% ($p > 0.05$), 8.25% ($p > 0.05$), 11.48% ($p > 0.05$) and 4.44% ($p > 0.05$), respectively, compared with CK, The order of chlorophyll a/b was $\text{G2} > \text{G3} > \text{G1} > \text{CK}$. It shows that grafting plant will affecting the photosynthetic rate, thus affecting the content of photosynthetic pigment, the chloroplast membrane of seedlings will be damaged under Cd stress, reducing the chloroplast photosynthetic enzyme content, causing leaf stomatal closure, obstructed photosynthesis, photosynthesis intensity decreases significantly, which leads to the decreased of chlorophyll content of plants, and grafting can improve the resistance of crops, such as low temperature, heavy metal stress.

Photosynthetic Characteristics of *L. esculentum* seedlings. Compared with the CK, The Pn of *L. esculentum* seedlings after grafted decreased to some extent under Cd stress, G1, G2 and G3 were decreased by 4.14% ($p < 0.05$), 4.89% ($p < 0.05$) and 11.78% ($p < 0.05$), it showed that grafting has

inhibitory effect on Photosynthetic rate. Leaf Gs sequence were ranked as CK > G2 > G3 > G1, leaf Ci sequence were ranked as CK > G1 > G2 > G3, showed that a large number of stomata are closed under Cd stress; G3 increased in leaves of Tr under Cd stress, increased by 1.54% ($p > 0.05$) compared to CK, the higher Pn can be decreasing leaf temperature, has a certain effect on alleviating under Cd stress, while Vpdl increased compared with CK, the G1 reached the highest, 53.11% ($p > 0.05$) higher than the CK.

Table 1 Photosynthetic pigment contents in *L. esculentum* seedlings

Treatments	Chlorophyll <i>a</i> (mg/g)	Chlorophyll <i>b</i> (mg/g)	Total chlorophyll (mg/g)	Chlorophyll <i>a/b</i>	Carotenoid (mg/g)
CK	1.579±0.006a	0.485±0.050a	2.064±0.044a	3.270	0.338±0.034a
G1	1.542±0.048a	0.445±0.020a	1.987±0.068a	3.464	0.323±0.003a
G2	1.541±0.132a	0.407±0.086a	1.948±0.046a	3.906	0.305±0.021a
G3	1.406±0.095a	0.421±0.048a	1.827±0.047a	3.376	0.268±0.011a

Values are means ± standard errors. Means with the same letter within each grafting times are not significantly different at $p < 0.05$.

Table 2 Photosynthetic characteristics of *L. esculentum* seedlings

Treatments	Pn ($\mu\text{mol CO}_2/\text{m}^2/\text{s}$)	Gs ($\text{mol H}_2\text{O}/\text{m}/\text{s}$)	Ci ($\mu\text{mol CO}_2/\text{mol}$)	Tr ($\text{mmol H}_2\text{O}/\text{m}^2/\text{s}$)	Vpdl (kPa)
CK	19.950±0.141a	0.385±0.109a	341.559±6.312a	4.356±0.873a	1.608±0.392a
G1	19.125±0.177ab	0.245±0.119a	329.198±9.709a	3.891±1.121a	2.462±1.038a
G2	18.975±0.106b	0.293±0.167a	253.437±1.031b	4.225±1.123a	1.939±1.118a
G3	17.600±0.212c	0.290±0.041a	244.152±0.434b	4.423±0.248a	1.690±0.080a

Values are means ± standard errors. Means with the same letter within each grafting times are not significantly different at $p < 0.05$.

Soluble Sugar Contents in *L. esculentum* seedlings. Compared with CK, the soluble sugar content of the *L. esculentum* seedlings roots, stems, leaves and shoots were increased significantly under Cd stress, G1, G2 and G3 increased the soluble sugar content in roots by 15.92% ($p > 0.05$), 9.91% ($p > 0.05$) and 2.67% ($p > 0.05$), the soluble sugar content in stems of *L. esculentum* seedlings were ranked as G1 > G3 > CK > G2, compared with CK, G1, G2 and G3 increased the soluble sugar content in leaves by 83.03% ($p < 0.05$), 50.26% ($p < 0.05$) and 27.77% ($p < 0.05$), and the soluble sugar content in shoots of *L. esculentum* seedlings were ranked as G1 > G2 > G3 > CK. This result revealed that the concentration of soluble sugar varied greatly at different grafted times, there was a reason that the stock incompatible with scion, the content of soluble sugar have a obviously decreased after continuous grafted.

Table 3 Soluble sugar contents in *L. esculentum* seedlings

Treatments	Roots (mg/g)	Stems (mg/g)	Leaves (mg/g)	Shoots (mg/g)
CK	79.215±3.029a	59.744±0.482b	42.400±0.593d	51.421±1.379c
G1	91.824±7.704a	75.364±1.832a	77.606±2.377a	76.577±2.060a
G2	87.067±6.530a	57.729±0.947b	63.710±0.659b	60.646±1.137b
G3	81.330±6.920a	60.751±5.363b	54.175±1.769c	57.447±3.364b

Values are means ± standard errors. Means with the same letter within each grafting times are not significantly different at $p < 0.05$.

Conclusions

Under Cd stress, G1, G2 and G3 decreased the chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid content of the *L. esculentum* seedlings compared with CK, and the total chlorophyll content of *L. esculentum* seedlings were ranked as CK > G1 > G2 > G3. The continuous grafted decreased the Pn, Gs and Ci of *L. esculentum* seedlings, and increased the Vpdl, and the Pn were ranked as CK > G1 > G2 > G3. Compared with CK, the continuous grafted increased the soluble sugar contents in roots, stems, leaves and shoots of *L. esculentum* seedlings, and the soluble sugar contents in roots and shoots were ranked as G1 > G2 > G3 > CK. Therefore, cutting after continuous grafted is unfavorable for the growth of the *L. esculentum* seedlings under Cd stress.

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