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Research Article

Effects of grafting on the morphology, physiology, and aerenchyma of balsam pear aboveground under waterlogging stress

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Abstract

The effects of grafting on the morphology, physiology, and aerenchyma of balsam pear aboveground under waterlogging stress were studied using a two-factor randomized block design. At 8 and 16 days, the degree of reduction of grafted balsam pear was lower than those of self-rooted balsam pear, although the height and leaf number of self-rooted and grafted balsam pears were remarkably reduced under waterlogging stress. Compared with self-rooted balsam pear, grafting considerably decreased the malondialdehyde content of balsam pear leaves but substantially increased the activities of antioxidant enzymes (superoxide dismutase, peroxidase, and catalase) and the contents of osmosis-regulating substances (soluble sugar, soluble protein, and proline) in the leaves of balsam pear under waterlogging stress at 4, 8, and 16 days. The stem of grafted balsam pear formed aerenchyma (pith cavity) at 0 days, whereas the stem of self-rooted balsam pear formed aerenchyma at 4 days. The aerenchyma of the stem formed by grafted balsam pear was more developed than that formed by the self-rooted balsam pear under waterlogging stress. The petiole of self-rooted and grafted balsam pears formed aerenchyma at 16 days, and the aerenchyma of grafted balsam pear was more developed than that of self-rooted balsam pear. These results indicated that grafting improved the antioxidant and osmotic regulation ability of balsam pear and enhanced the tolerance of balsam pear to waterlogging stress by enlarging the pith cavity of the stem and petiole of balsam pear.

Keywords: aerenchyma; antioxidant enzyme activity; grafted bitter melon; osmosis-regulating substances; waterlogged substrate

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Introduction

Waterlogging stress is the most common abiotic stress and leads to considerable morphology and physiological modifications of plants during their growth stage (Zhang *et al.*, 2019; Peng *et al.*, 2019). Waterlogging stress inhibits the growth of some plants, such as *Cucumis sativus* L. and *Saccharum officinarum*

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L. (Tavares *et al*., 2018; Barickman *et al*., 2019). Waterlogging stress can damage plants but can be alleviated through a series of responses (Chávez-Arias *et al*., 2019; Wang *et al*., 2019). Reactive oxygen species (ROS), including O_2^-, H_2O_2, and HO^-, are toxic by-products of cellular aerobic metabolism (Guo *et al*., 2018). Waterlogging stress can increase ROS contents (Duan *et al*., 2019), which are harmful to the biofilm system of plants because ROS can destroy normal metabolism through oxidative damage of lipids, proteins, deoxyribonucleic acids, and carbohydrates (Kavas *et al*., 2013). Antioxidant enzyme activity is an important indicator of plant resistance because antioxidants can effectively remove ROS (Foyer *et al*., 2018; Gong *et al*., 2018). Waterlogging stress can increase the enzyme activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) and thus cause ROS scavenging (Li *et al*., 2018; Alizadeh-Vaskasi *et al*., 2018). Water potential in plant leaves decreases under waterlogging stress, but some plants, such as *Robinia pseudoacacia* L. and *Triticum aestivum* L. (Yang *et al*., 2018; Khosravi *et al*., 2018), can maintain the normal morphology of leaves by accumulating osmotic substances. Moreover, the stems and petioles of some plants, such as *Cynodon dactylon* and ferns (Yang *et al*., 2011; Barton *et al*., 2015), can also form aerenchyma (pith cavity) to adapt to waterlogged environments. Furthermore, grafting, which is an extensively used technique for vegetable production (Al-Harbi *et al*., 2018; Miao *et al*., 2019), can be used to improve the resistance of crops to abiotic stresses, such as salt and cold stresses (Madadkhah *et al*., 2018; Suchoff *et al*., 2018).

Balsam pear (*Momordica charantia* L.) is an important melon vegetable that is rich in saponins, which have medicinal values, such as lowering blood glucose and antiviral properties (Perez *et al*., 2019). The root growth of balsam pear is poor and yield is greatly reduced in waterlogged areas. *Luffa cylindrica* (L.) Roem is also an important melon vegetable, which can grow well in soil with moisture content more than 70% (Amrina *et al*., 2019). In this experiment, we studied the growth and adaptation mechanism of balsam pear grafted on towel gourd under waterlogging stress.

Materials and Methods

Experiment materials and design

The experiment was conducted in a glass greenhouse at Yangtze University, Jingzhou City (30° 21′ N, 112° 8′ E) in Hubei Province, China from April to August 2019. Towel gourd variety cv. "Zaojia" seeds were provided by Zhuzhou Nongzhi Co., Ltd., and balsam pear variety cv. "Hualvzhuangyuan" seeds were provided by the Chunhua Seed Industry Center, Yichun City, Jiangxi Province. The cultivation substrate was provided by Jiangsu Peilei Matrix Technology Development Co., Ltd. A two-factor randomized block design was used in the experiment. Factor 1 included the type of propagation (self-rooted vs. grafted), and Factor 2 was whether or not the plants were subjected to waterlogging. Four treatments and three biological replications were conducted.

Plant treatment

When the towel gourds seedlings were unearthed, the balsam pears were sprouted. When the balsam pear seedlings were unearthed, some of the balsam pears were grafted onto towel gourds through insertion method, and some balsam pears were self-rooted. Towel gourds were transplanted into culture pots (20 cm in diameter, 15 cm in height) when the grafted balsam pear had four leaves and one heart. For the waterlogged treatment, the seedlings were transplanted for 7 days and then waterlogged (water level was kept at 1 cm below the cotyledon). All morphological and physiological indicators were measured via random sampling at 0, 1, 2, 4, 8, and 16 days of waterlogging.

Determination of growth parameters

Plant height was measured by using a tapeline and the number of leaves of each plant were counted at 0, 1, 2, 4, 8, and 16 days.

Determination of lipid peroxidation, enzyme activity, and osmosis-adjusting-substance content

Malondialdehyde (MDA) content was measured based on the method of Esterbauer and Cheeseman (1990). SOD activity was estimated through the method of Beauchamp and Fridovich (1971), POD activity was estimated through the method of Prochazkova *et al.* (2001), and CAT activity was estimated by using the method of Chance and Maehly (1955). Soluble sugar was estimated through the method of Yemm and Willis (1954). Soluble proteins were estimated per the method of Bradford (1976). Content of proline were determined per the method of Li (2000).

Observation of aerenchyma of stem and petiole

Stem (1 cm below the cotyledon) and petiole (1 cm away from the leaf base) were sectioned through freehand, stained with toluidine blue O, and observed under an anatomic microscope (Yang *et al.*, 2011).

Statistical analysis

All data were analysed with SAS 9.1 software, and Duncan's new complex range method was used to compare significant differences.

Results

Plant height

The heights of grafted and self-rooted balsam pears were remarkably reduced under waterlogging at 8 and 16 days, but the degree of reduction of the growth of grafted balsam pear was lower than that of the self-rooted balsam pear ($P < 0.05$) (Table 1). The height of grafted and self-rooted balsam pears decreased by 19.4% and 20.2%, respectively, at 8 days of waterlogging and by 26.6% and 27.0%, respectively, at 16 days of waterlogging compared with non-waterlogged plants.

Number of leaves

The number of leaves of grafted and self-rooted balsam pears was remarkably reduced at 8 and 16 days after waterlogging, and the degree of the reduction of the number of leaves of grafted balsam pear was lower than that of the self-rooted balsam pear ($P < 0.05$) (Table 2). The number of leaves of grafted and self-rooted balsam pears decreased by 13.7% and 17.1%, respectively, after 8 days of waterlogging and by 15.5% and 23.1%, respectively, after 16 days of waterlogging compared with non-waterlogged plants.

Lipid peroxidation

The MDA contents of grafted and self-rooted balsam pears significantly increased ($P < 0.05$) during the 16 days of waterlogging (Figure 1). At 1–16 days, grafting significantly reduced the MDA content of the leaves of waterlogged and non-waterlogged balsam pears ($P < 0.05$). MDA content in grafted balsam pear leaves at 1, 2, 4, 8, and 16 days decreased by 40.9%, 40.5%, 38.7%, 40.8% and 41.7% without waterlogging and decreased by 41.6%, 45.6%, 52.1%, 54.9%, and 53.0% under waterlogging, respectively, compared with self-rooted balsam pear.

Table 1. Plant height of grafted balsam pear under waterlogging stress

	Treatment time, d					
	0	1	2	4	8	16
M/M + NW	22.33±3.35a	31.73±1.08a	35.47±4.89a	39.67±5.80a	69.57±5.95a	104.57±6.38a
M/M + W	21.70±2.84a	30.10±1.10a	33.10±4.44a	35.73±4.35a	51.03±6.92b	76.30±4.78b
M/L + NW	10.70±2.61b	11.37±2.09b	13.13±3.15b	18.27±2.52b	37.70±6.31c	82.27±8.71b
M/L + W	9.93±2.35b	10.60±1.78b	11.20±3.38b	15.30±2.05b	30.37±5.28d	65.67±4.24c
Analysis of variance						
Grafting (G)	**	**	**	**	**	**
Waterlogging (W)	ns	ns	ns	ns	**	**
G x W	ns	ns	ns	ns	**	ns

M: *Momordica charantia*, L: *Luffa cylindrica*, NW: no waterlogged substrate, W: waterlogged substrate. Data were shown as means ± SE. Different letters in the table indicate significant differences between treatments at the same time ($P < 0.05$).

Table 2. Number of leaves of grafted balsam pear under waterlogging stress

	Treatment time, d					
	0	1	2	4	8	16
M/M + NW	6.0±0.0a	7.0±0.0a	8.0±0.0a	9.0±0.6a	11.0±0.6a	16.0±1.2a
M/M + W	5.7±0.3a	6.7±0.3a	7.7±0.3a	8.3±0.7a	9.3±0.3b	12.3±0.9b
M/L + NW	4.7±0.3b	5.0±0.0b	5.7±0.3b	6.0±0.6b	7.3±0.9c	11.7±0.7b
M/L + W	4.3±0.3b	4.7±0.3b	5.3±0.3b	5.7±0.3b	6.3±0.3d	9.7±0.7c
Analysis of variance						
Grafting (G)	**	**	**	**	**	**
Waterlogging (W)	ns	ns	ns	*	**	**
G×W	ns	ns	ns	ns	ns	*

M: *Momordica charantia*, L*: Luffa cylindrica*, NW: no waterlogged substrate, W: waterlogged substrate. Data were shown as means ± SE. Different letters in the table indicate significant differences between treatments at the same time ($P < 0.05$).

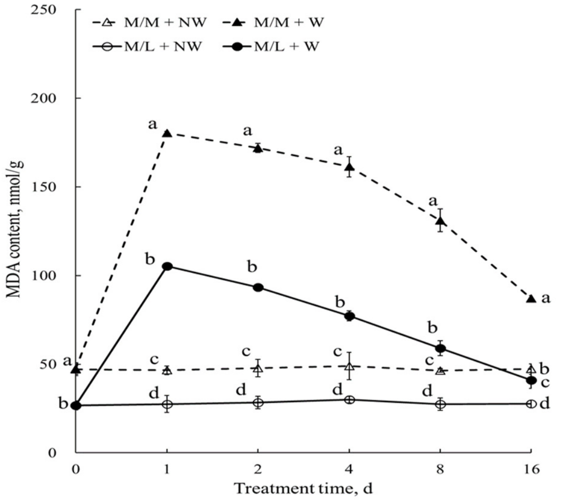

Figure 1.MDA content in leaves of grafted balsam pear under waterlogging stress
M: *Momordica charantia*, L: *Luffa cylindrica*, NW: no waterlogged substrate, W: waterlogged substrate. Data were shown as means ± SE. Different letters in the table indicate significant differences between treatments at the same time ($P < 0.05$).

SOD, POD, and CAT activities

The SOD, POD and CAT activities of grafted and self-rooted balsam pears were significantly increased ($P < 0.05$) at 4, 8, and 16 days of waterlogging (Figure 2(a), 2(b), 2(c)). The SOD activity of grafted balsam pear 4, 8, and 16 days of waterlogging remarkably increased by 6.0%, 16.6%, and 16.7%, respectively, compared with self-rooted balsam pear (Figure 2(a)). Grafting significantly increased the POD activity in the leaves of waterlogged and non-waterlogged balsam pears ($P < 0.05$) at 4, 8, and 16 days (Figure 2(b)). The POD activity of grafted balsam pear leaves at 4, 8, and 16 days increased by 153.3%, 153.1% and 152.9% without waterlogging and increased by 167.5%, 292.3%, and 207.3% under waterlogging, respectively, compared with self-rooted balsam pear. The CAT activity of grafted balsam pear leaves at 2, 4, 8, and 16 days of waterlogging substantially increased by 18.4%, 16.8%, 14.0%, and 25.9%, respectively, compared with self-rooted balsam pear (Figure 2(c)).

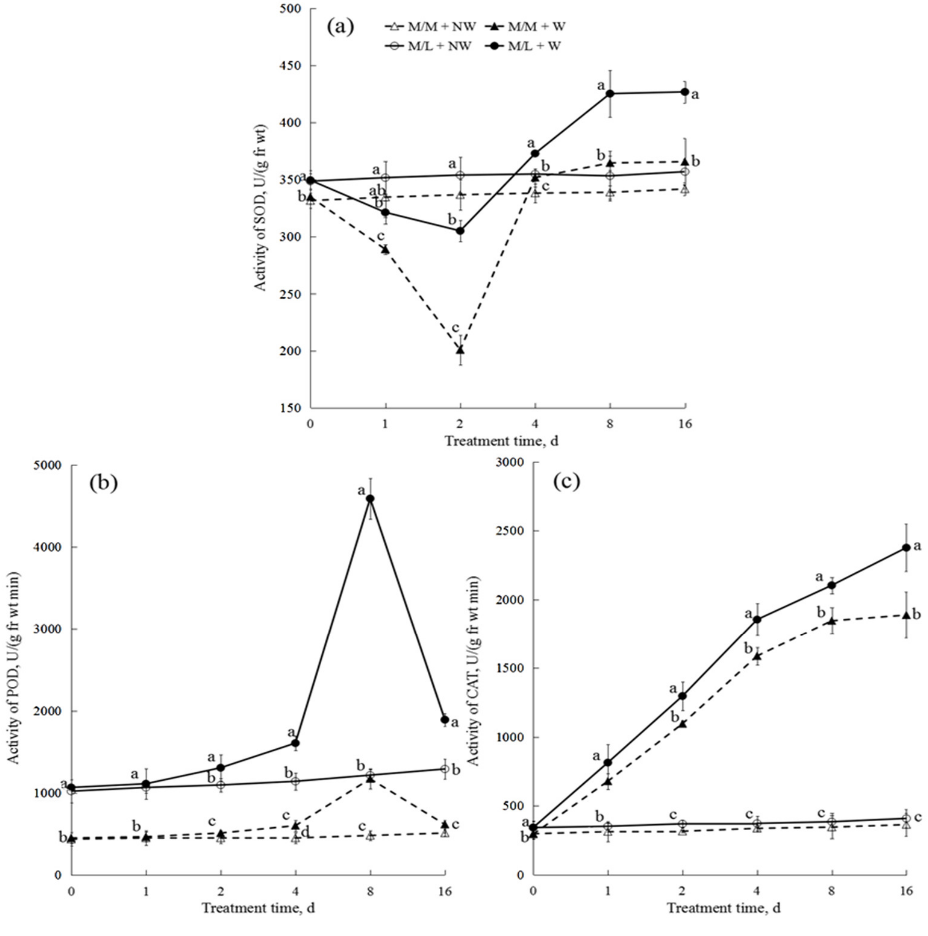

Figure 2. Antioxidant enzyme activity in leaves of grafted balsam pear under waterlogging stress

M: *Momordica charantia*, L: *Luffa cylindrica*, NW: no waterlogged substrate, W: waterlogged substrate. Data were shown as means ± SE. Different letters in the table indicate significant differences between treatments at the same time ($P < 0.05$)

Soluble sugar, soluble protein, and free proline content

The soluble sugar contents of grafted and self-rooted balsam pears were significantly increased ($P < 0.05$) at 2, 4, 8, and 16 days of waterlogging (Figure 3(a)). Grafting significantly increased ($P < 0.05$) the soluble sugar content in leaves under waterlogged and non-waterlogged conditions at 2, 4, 8, and 16 days. The soluble sugar content of grafted balsam pear at 2, 4, 8, and 16 days increased by 21.6%, 18.5%, 12.6% and 12.3%, respectively, without waterlogging and increased by 24.5%, 27.5%, 27.7% and 17.3%, respectively, under waterlogging compared with self-rooted balsam pear. The soluble protein contents of grafted and self-rooted balsam pears were significantly increased (P < 0.05) at 4, 8, and 16 days of waterlogging (Figure 3(b)). Grafting considerably increased (P < 0.05) the soluble protein content in leaves waterlogged and non-waterlogged conditions at 4, 8, and 16 days. The soluble protein content of grafted balsam pear at 4, 8, and 16 days increased by 7.1%, 9.5%, and 8.0%, respectively, without waterlogging and increased by 13.2%, 16.4%, and 10.8%, respectively, under waterlogging compared with self-rooted balsam pear. The proline contents of grafted and self-rooted balsam pears were significantly increased (P < 0.05) at 1, 2, and 4 days of waterlogging (Figure 3(c)). The proline content of grafted balsam pear leaves at 1, 2, 4, 8, and 16 days increased considerably by 16.1%, 15.2%, 8.2%, 17.6%, and 6.3%, respectively, compared with self-rooted balsam pear.

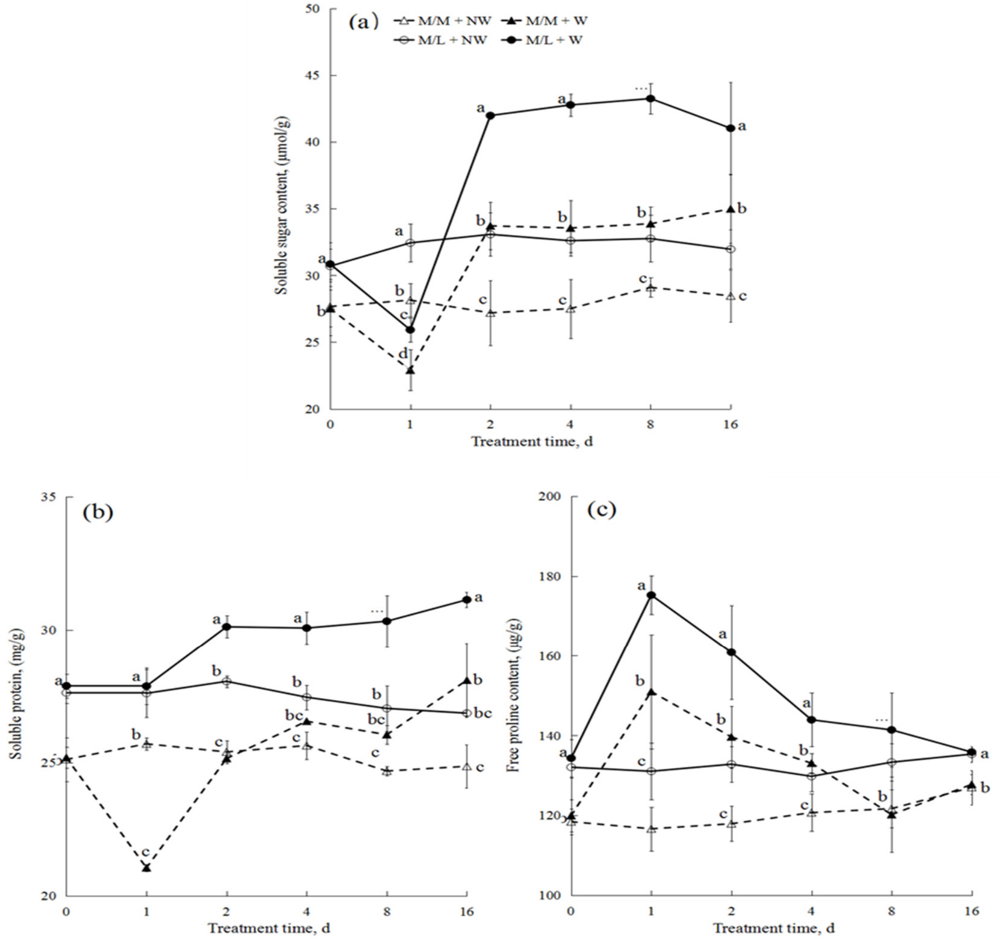

Figure 3. Osmotic adjustment substances content in leaves of grafted balsam pear under waterlogging stress
M: *Momordica charantia*, L: *Luffa cylindrica*, NW: no waterlogged substrate, W: waterlogged substrate. Data were shown as means ± SE. Different letters in the table indicate significant differences between treatments at the same time (P < 0.05)

Aerenchyma of the stem

At 0–2 days, the stem of grafted balsam pear formed a pith cavity under waterlogged and non-waterlogged conditions (Figure 4). At 4–16 days, the stems of grafted and self-rooted balsam pears formed a pith cavity under waterlogged and non-waterlogged conditions, and the pith cavity of grafted balsam pear stem was more developed than that of self-rooted balsam pear.

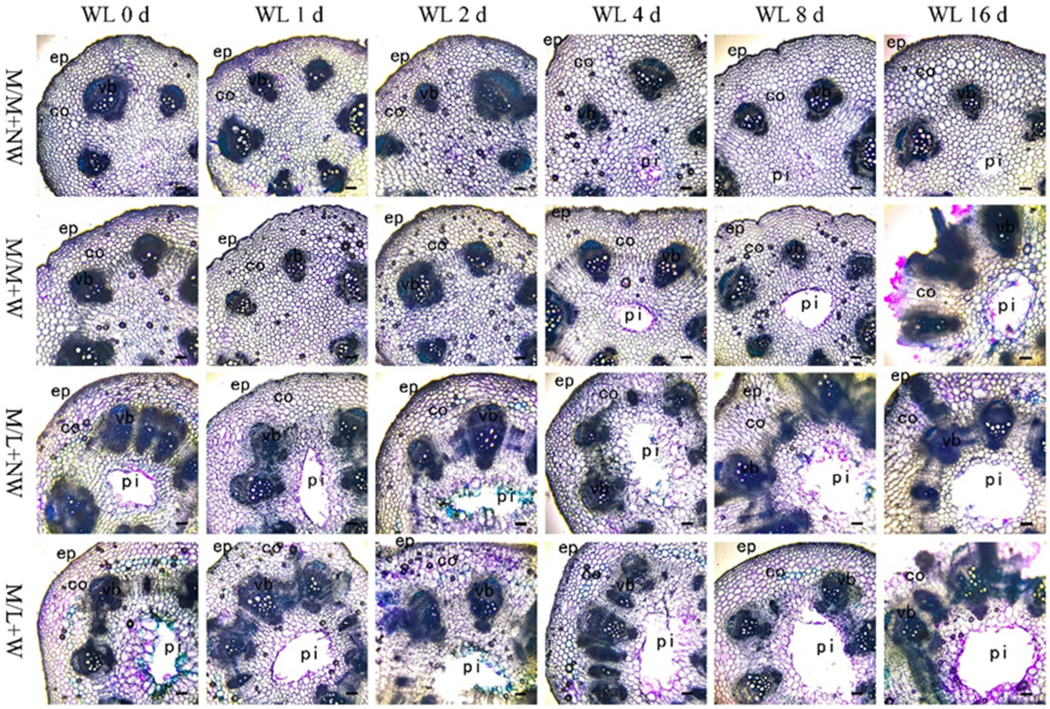

Figure 4. Effect of waterlogging stress on aerenchyma of grafted balsam pear stem
Ep, epidermis; Co, cortex; Vb, vascular bundle; Pi, pith cavity. Ruler =125 μm.

Aerenchyma of the petiole

The petiole of self-rooted and grafted balsam pears had formed pith cavity at 16 days of waterlogging, and the pith cavity of the petiole of grafted balsam pear was more developed than that of self-rooted balsam pear (Figure 5).

Discussion

Plant height and leaf number are important indicators of plant growth. In this experiment, waterlogging remarkably reduced the height and leaf number of grafted balsam pear at 8 and 16 days. This result indicated that waterlogging inhibits the growth of grafted balsam pear similarly to *Cucumis sativus* L. and *Saccharum officinarum* L. (Tavares *et al*., 2018; Barickman *et al*., 2019). This finding is attributed to the inhibition of anaerobic respiration of roots during waterlogging stress, in which the roots do not have enough energy to absorb nutrients and maintain the normal growth of grafted balsam pear aboveground. Grafting substantially reduced the height and leaf number of balsam pear under waterlogged and non-waterlogged conditions because the growth rate of self-rooted balsam pear was faster than grafted balsam pear during the healing period of grafting. The degrees of reduction of the height and leaf number of grafted balsam pear at 8 and 16 days were lower than self-rooted balsam pear under waterlogging. This result suggested that the waterlogging resistance of grafted balsam pear is better than that of self-rooted balsam pear possibly because the roots of the host of grafted balsam pear was more developed than self-rooted balsam pear, and the ability of the host of grafted balsam pear to absorb nutrients was stronger than self-rooted balsam pear.

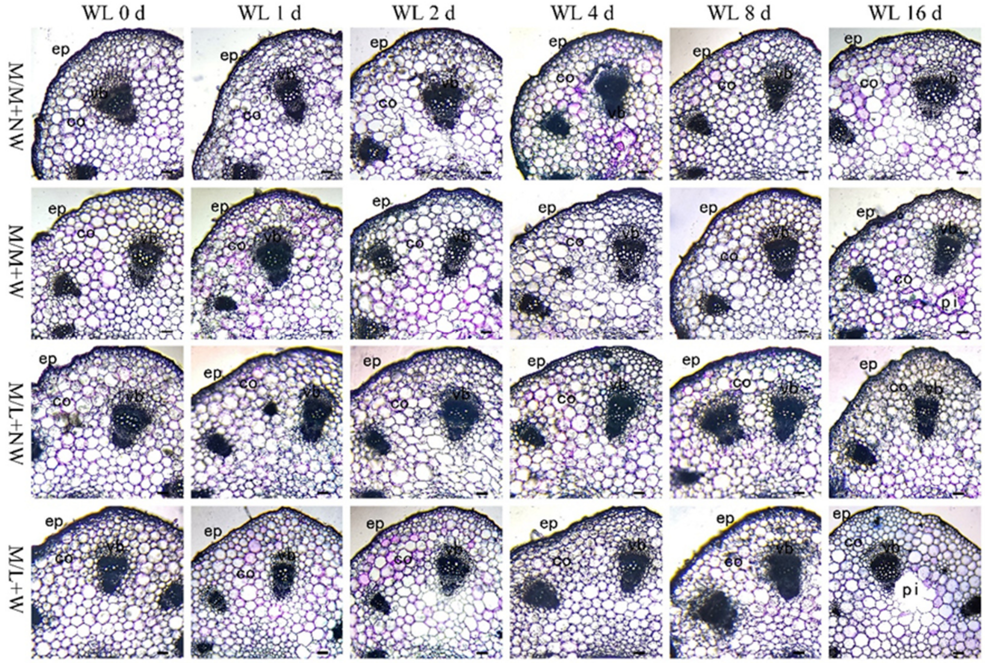

Figure 5. Effect of waterlogging stress on aerenchyma of grafted balsam pear petiole
Ep, epidermis; Co, cortex; Vb, vascular bundle; Pi, pith cavity. Ruler =125 μm.

MDA is one of the peroxidation products of lipid, and its content can reflect the degree of damage to plants. In this experiment, waterlogging stress significantly increased the MDA content of grafted balsam pear at 1–16 days because ROS content increased and antioxidant enzymes cannot scavenge ROS under waterlogging. MDA content in the leaves of grafted balsam pear was remarkably lower than that of self-rooted balsam pear at 1–16 days. This result suggests that grafting reduced the degree of lipid peroxidation of balsam pear because the SOD, POD, and CAT activities of grafted balsam pear were higher than those of self-rooted balsam pear. The MDA content of grafted balsam pear decreased initially and then increased under waterlogging at 0–16 days. This finding was attributed to the increase in CAT activity after 1 day of waterlogging and the ROS-scavenging ability of SOD, POD, and CAT was weak. The activities of SOD, POD, and CAT were considerably increased at 4 days, and their ability to scavenge ROS was strong. SOD can catalyse the conversion of superoxide anions into H_2O_2 and O_2, and POD and CAT can catalyse the decomposition of H_2O_2 into H_2O and O_2 (Figure 6). In this experiment, the activities of SOD, POD, and CAT of grafted balsam pear were substantially increased under waterlogged treatment at 4 days compared with non-waterlogged treatment. This finding indicates that grafted balsam pear can resist waterlogging stress by increasing the activities of antioxidant enzymes. The activities of SOD, POD, and CAT of grafted balsam pear were significantly higher than those of self-rooted balsam pear at 4–16 days of waterlogging. This result suggests that grafting improved the antioxidant capacity of balsam pear. The SOD activity of grafted balsam pear initially decreased and then increased during the 16 days of waterlogging. This finding suggests that the leaves of grafted balsam pear accumulated large amounts of H_2O_2, which inhibit the activity of SOD after 1 day of waterlogging. POD and CAT reduced some of the H_2O_2 at 2–16 days of waterlogging, and the SOD activity of grafted balsam pear was no longer inhibited and thus increased rapidly. This result is different from those observed in *Zea mays* L. and *Triticum aestivum* L. probably due to different test conditions and materials used (Li *et al.*, 2018; Alizadeh-vaskasi *et al.*, 2018).

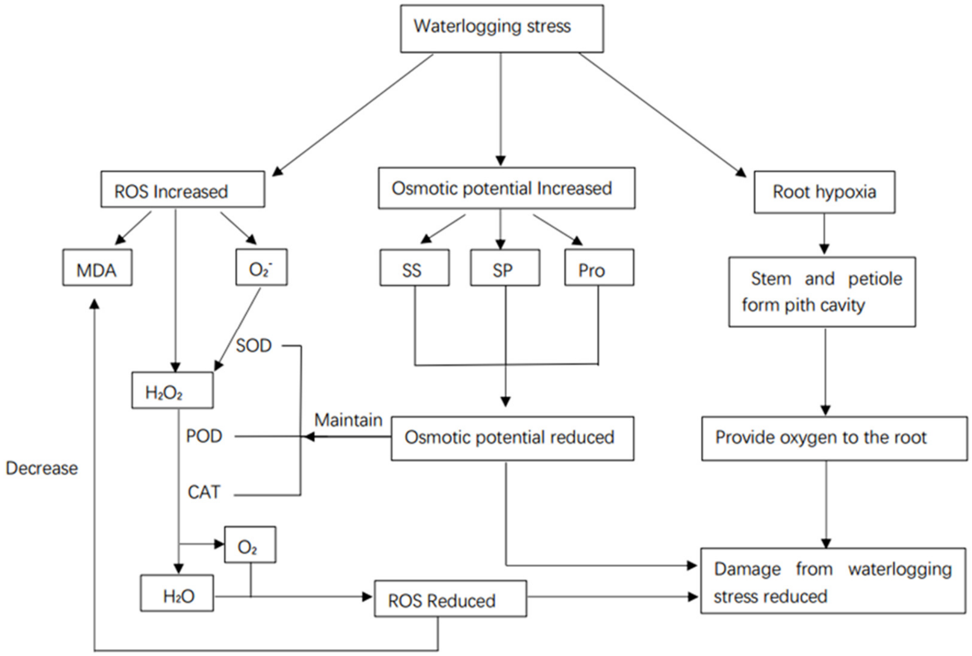

Figure 5. Mechanism of the grafted balsam pear aboveground adapted to the waterlogging stress
SS, soluble sugar; SP, soluble protein; Pro, proline

Plants enhance their stress resistance by accumulating considerable amounts of soluble sugar, soluble protein, and proline to improve cell sap concentration, which can maintain cell turbidity and prevent excessive plasma dehydration (Guo *et al.*, 2018), thereby stabilizing their intracellular macromolecular structure and maintaining enzyme activity (Figure 6). In this experiment, the soluble sugar, soluble protein, and proline contents of grafted balsam pear were remarkably increased under waterlogged treatment at 4 days compared with non-waterlogged treatment. This result suggests that grafted balsam pear can resist waterlogging stress by accumulating osmotic substances. Soluble sugar, soluble protein, and proline in grafted balsam pear were remarkably higher than those in self-rooted balsam pear at 4 days of waterlogging. This finding indicates that grafting improved the osmosis-adjustment ability of balsam pear under waterlogging. At 0–16 days of waterlogging, the contents of soluble sugar and soluble protein of grafted balsam pear initially decreased and then increased, and proline content initially increased and then decreased. This outcome indicated that the cell turbidity of grafted balsam pear was maintained by accumulating proline first and then obtaining soluble sugar and protein. This finding differs from those observed in *Robinia pseudoacacia* L. and *Triticum aestivum* L. probably because of differences in methods, test materials, and environment (Yang *et al.*, 2018; Khosravi *et al.*, 2018).

The formation of aerenchyma is an important plant adaptation to waterlogged environment. Some plants adapt to waterlogged environment by forming aerenchyma in their stems and petioles. In this experiment, the stem and petiole of grafted and self-rooted balsam pears formed aerenchyma at 16 days of waterlogging. The adventitious roots of grafted balsam pear formed aerenchyma under waterlogging stress (Peng *et al.*, 2019). This finding indicates that stems and petioles can form a channel, which is connected with the aerenchyma of roots, to transport and store oxygen and to provide oxygen from the inside to the roots, thus relieving hypoxia stress caused by waterlogging on the roots. This phenomenon is similar to those observed in *Cynodon dactylon* and ferns (Yang *et al.*, 2011; Barton *et al.*, 2015). The stem of grafted balsam pear had formed pith cavity at 0 day of waterlogging, whereas the stem of self-rooted balsam pear had not formed pith cavity until 4 days of waterlogging. This outcome indicated that grafted balsam pear already had the morphological characteristics to adapt to waterlogging stress after grafting., the pith cavity in the stem and petiole of balsam pear was expanded by grafting at 16 days of waterlogging. This result suggests that the roots of grafted balsam pear is more developed than self-rooted balsam pear and needs more oxygen. Moreover, the lack of oxygen to stimulate grafted balsam pear caused the formation of more ethylene and stronger cellulase activity than self-rooted grafted pear to dissolve more cortical cells.

Conclusions

Overall, waterlogging stress inhibited the growth of grafted balsam pear aboveground, whereas grafting increased the resistance of balsam pear to waterlogging stress by (1) increasing the activity of antioxidant enzymes in the leaves of balsam pear, (2) increasing the content of osmosis-adjusting substances in the leaves of balsam pear, and (3) expanding the aerenchyma of the stem and petiole of balsam pear.

Authors' Contributions

Literature search, Data acquisition, Manuscript editing: WJ L.
Data acquisition, Data analysis and statistical analysis: MH Y, LT X and YQ P.
The concepts, design and performed manuscript review: J Z.
All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Alizadeh-vaskasi F, Pirdashti H, Araei AC, Saadatmand S (2018). Waterlogging effects on some antioxidant enzymes activities and yield of three wheat promising lines. Acta Agriculturae Slovenica 111(3):621-631. *https://doi.org/10.14720/aas.2018.111.3.10*

Amrina H, Shahzad S, Siddiqui ZS (2019). Photochemistry of *Luffa cylindrica* (L.) Roem under fungal biocontrol interaction. Photosynthetica 56(2):743-749. *https://doi.org/10.1007/s11099-017-0729-9*

Al-Harbi AR, Al-Omran AM, Alharbi K (2018). Grafting improves cucumber water stress tolerance in Saudi Arabia. Saudi Journal of Biological Sciences 25(2):298-304. *https://doi.org/10.1016/j.sjbs.2017.10.025*

Beauchamp C, Fridovich I (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry 44(1):276-287. *https://doi.org/10.1016/0003-2697(71)90370-8*

Barton DA, Overall RL, Thomson JA (2015). Structure and development of the lateral-line aerenchyma in bracken ferns (Pteridium: Dennstaedtiaceae). International Journal of Plant Sciences 176(7):662-669. *https://doi.org/10.1086/682055*

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Analytical Biochemistry 72(1):248-254. *https://doi.org/10.1016/0003-2697(76)90527-3*

Barickman TC, Simpson CR, Sams CE (2019). Waterlogging causes early modification in the physiological performance, carotenoids, chlorophylls, proline, and soluble sugars of cucumber plants. Plants 8(6):160-174. *https://doi.org/10.3390/plants8060160*

Chávez-Arias CC, Gómez-Caro S, Restrepo-Díaz H (2019). Physiological, biochemical and chlorophyll fluorescence parameters of *Physalis peruviana* L. seedlings exposed to different short-term waterlogging periods and *Fusarium* wilt infection. Agronomy 9(5):213. *https://doi.org/10.3390/agronomy9050213*

Chance B, Maehly AC (1954). The assay of catalases and peroxidases. Methods of Biochemical Analysis 76(21):764-775. *https://doi.org/10.1002/9780470110171.ch14*

Duan XX, Qin D, Song HC, Gao TC, Zuo SH, Yan X, Dong JY (2019). Irpexlacte AD, four new bioactive metabolites of endophytic fungus *Irpex lacteus* DR10-1 from the waterlogging tolerant plant *Distylium chinense*. Phytochemistry Letters 32:151-156.

Esterbauer HK, Cheeseman H (1990). Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. Methods in Enzymology 186:407-421. *https://doi.org/10.1016/0076-6879(90)86134-h*

Foyer CH (2018). Reactive oxygen species, oxidative signaling and the regulation of photosynthesis. Environmental and Experimental Botany 154:134-142. *https://doi.org/10.1016/j.envexpbot.2018.05.003*

Gong DH, Wang GZ, Si WT, Zhou Y, Liu Z, Jia J (2018). Effects of salt stress on photosynthetic pigments and activity of ribulose-1,5-bisphosphate carboxylase/oxygenase in *Kalidium foliatum*. Russian Journal of Plant Physiology 65(1):98-103. *https://doi.org/10.1134/S1021443718010144*

Guo YY, Yu HY, Yang MM, Kong DS, Zhang YJ (2019). Effect of drought stress on lipid peroxidation, osmotic adjustment and antioxidant enzyme activity of leaves and roots of *Lycium ruthenicum* Murr. seedling. Russian Journal of Plant Physiology 65(1):244-250. *https://doi.org/10.1134/S1021443718020127*

Kavas M, Baloglu MC, Akca O, Köse FS, Gökçay D (2013). Effect of drought stress on oxidative damage and antioxidant enzyme activity in melon seedlings. Turkish Journal of Biology 37:491-498. *https://doi.org/10.3906/biy-1210-55*

Khosravi M S, Heidari R, Jamei R, Kouhi SMM, Moudi M (2018). Comparative growth and physiological responses of tetraploid and hexaploid species of wheat to flooding stress. Acta Agriculturae Slovenica 111(2):285-292. *https://doi.org/10.14720/AAS.2018.111.2.04*

Li HS (2000). Principle and techniques of botanic, chemical and physiological experiments. Bejing: Higher Education Press.

Li W, Mo W, Ashraf U, Li G, Wen T, Abrar M, Hu J (2018). Evaluation of physiological indices of waterlogging tolerance of different maize varieties in South China. Applied Ecology Environmental Research 16(18):2059-2072. *https://doi.org/10.15666/aeer/1602_20592072*

Madadkhah E, Bolandnazar S, Oustan S (2018). Effect of salt stress on growth, antioxidant enzymes activity, lipid peroxidation and photosystem II efficiency in cucumber grafted on cucurbit rootstock. Iranian Journal of Horticultural Sciences 49:465-475. *https://doi.org/10.22059/IJHS.2017.232193.1247*

Miao L, Li S, Bai L, Anwar A, Li Y, He C, Yu X (2019). Effect of grafting methods on physiological change of graft union formation in cucumber grafted onto bottle gourd rootstock. Scientia Horticulturae 244: 249-256. *https://doi.org/10.1016/j.scienta.2018.09.061*

Prochazkova D, Sairam RK, Srivastava GC, Singh DV (2001). Oxidative stress and antioxidant activity as the basis of senescence in maize leaves. Plant Science 161(4):765-771. *https://doi.org/10.1016/S0168-9452(01)00462-9*

Perez JL, Jayaprakasha GK, Patil BS (2019). Metabolite profiling and *in vitro* biological activities of two commercial balsam pear (*Momordica charantia* Linn.) cultivars. Food Chemistry 288:178-186. *https://doi.org/10.1016/j.foodchem.2019.02.120*

Peng, YQ, Zhu J, Li WJ, Gao W, Shen RY, Meng LJ (2019). Effects of grafting on root growth, anaerobic respiration enzyme activity and aerenchyma of bitter melon under waterlogging stress. Scientia Horticulturae 261:108977. *https://doi.org/10.1016/j.scienta.2019.108977*

Suchoff DH, Perkins-Veazie P, Sederoff HW, Schultheis JR, Kleinhenz MD, Louws FJ, Gunter CC (2018). Grafting the indeterminate tomato cultivar Moneymaker onto multifort rootstock improves cold tolerance. HortScience 53(11):1610-1617. *https://doi.org/10.21273/hortsci13311-18*

Tavares ACS, Duarte SN, da Silva Dias N, da Silva Sá FV, de Miranda JH, de Souza KTS, and dos Santos Fernandes C (2018). Growth of sugar cane under cultivation flooded at different speeds lowering of the water table. Journal of Agricultural Science 10(11):122-131. *https://doi.org/10.5539/jas.v10n10p122*

Wang H, Chen Y, Hu W, Snider JL, Zhou Z (2019). Short-term soil-waterlogging contributes to cotton cross tolerance to chronic elevated temperature by regulating ROS metabolism in the subtending leaf. Plant Physiology and Biochemistry 139(19): 333-341. *https://doi.org/10.1016/j.plaphy.2019.03.038*

Yang B, Peng C, Harrison S, Wei H, Wang H, Zhu Q, Wang M (2018). Allocation mechanisms of non-structural carbohydrates of *Robinia pseudoacacia* L. seedlings in response to drought and waterlogging. Forests 9(12):754. *https://doi.org/10.3390/f9120754*

Yang C, Zhang X, Zhou C, Seago Jr JL (2011). Root and stem anatomy and histochemistry of four grasses from the Jianghan Floodplain along the Yangtze River, China. Flora-Morphology, Distribution, Functional Ecology of Plants 206(7):653-661. *https://doi.org/10.1016/j.flora.2010.11.011*

Yemm EW, Willis AJ (1954). The estimation of carbohydrates in plant extracts by anthrone. Biochemical Journal 57(3):508-514. *https://doi.org/10.1042/bj0570508*

Zhang J, Yin DJ, Fan SX, Li SG, Dong L (2019) Modulation of morphological and several physiological parameters in sedum under waterlogging and subsequent drainage. Russian Journal of Plant Physiology 66:290-298. *https://doi.org/10.1134/S1021443719020183*