Defense Response of Pumpkin Rootstock To Cadmium

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Abstract

Cadmium pollution is severe in cucumber, although grafting is an effective method to improve its stress tolerance. Pumpkin is the commonly-used grafting rootstock for cucumber, and the breeding of rootstock with cadmium tolerance plays a vital role in the safe production of cucumber. However, there are no reports on rootstocks specific for cadmium tolerance. In this study, the rootstock of a pumpkin cross combination and its parents were used for the study of cadmium stress. The results indicated that under the 24mg·L^{-1}cadmium stress, the relative conductivity of cross combination decreased by 35.86%~36.31% compared with the parents. When the concentrations of cadmium stress were 8 mg·L^{-1}and 16 mg·L^{-1}, respectively, the peroxidase (POD) activity of cross combination was higher than those of the parents. The subcellular distribution of cadmium in the root systems of the cross and the 041 – 1 parent was in the cell wall first, followed by the cytoplasm and organelle, while that in the root system of 360-3 parent was in the cell wall first, followed by the organelle and cytoplasm. Under cadmium stress with the 24mg·L^{-1}concentration, the transfer coefficient of cross was significantly lower than that of the parents. The cross initiated the activity of membrane protective enzyme POD under cadmium stress, relieved the damage to membrane, and reduced the toxicity of cadmium through the accumulation of cadmium in the cell wall that blocked its entrance to the cytoplasm. This study provides a theoretical foundation to breed cadmium-tolerant rootstocks for melon vegetables.

Introduction

As one of the eight most toxic heavy metals in soil pollution, cadmium has a half-life as long as 10 ~ 35 days, which cannot be degraded by microorganisms and exists in soil for long time (Li et al.2017; Zheng et al.2006). Previous research (Shang et al.2018) indicated that the point-location standard-exceeding ratio of heavy metals in the arable soil of five major grain producing areas in China reached as high as21.49%, whereas the increase in proportion of Cd pollution was the most significant. It increased from 1.32–17.39% during twenty some years. Recently, greenhouse vegetables have been the leading industry for farmers. Chen et al.(Chen et al.2012a) found that the cadmium pollution in soil of greenhouse vegetable fields in Xinxiang city had reached as high aslevel6, which had already been severely polluted. The soil of vegetable fields in the regions that included Beijing (Xu et al.2017; Suo et al.2016), Nanjing(Chen et al. 2013) and Hebei(Zhao et al. 2019) were polluted by heavy metals to differing extents, whereas the cadmium pollution was most serious. Cadmium-polluted soil can not only affect the normal growth of crops, which leads to a decrease in crop yields and quality but can also be absorbed by the human body through food chain, which results in a serious threat to national food safety and human health.

Cucurbit vegetables are not only nutritious and delicious but also improve the diet and human health (XIA et al. 2010). However, when heavy metals are severe or exceed the standard for cultivated soil, the edible parts of melons easily accumulate heavy metals (Wang et al. 2018). The content of cadmium in cucumbers in China has reached as high as0.43mg·kg^{-1}(Li et al. 2017), far higher than the limiting standard of cadmium in vegetables (0.2mg·kg^{-1}), which is regulated in the National Food Safety Standards (GB2672-2017). Research (Youssef et al. 2008; Dimttrios et al. 2013) indicated that grafting could relieve the toxicity of heavy metals to the aboveground parts to some extent. The effects of grafting using different kinds of rootstocks on the content of cadmium in watermelon fruit (Huang et al. 2013) indicated that when using four different kinds of rootstocks, including cucurbit, wild watermelon, China pumpkin and India-China hybrid pumpkin, to graft watermelon, the content of cadmium in fruits of grafted seedlings through the use of China pumpkin and India-China hybrid pumpkin as rootstocks was the lowest, while in roots, it was the highest. Pumpkin was used as the common rootstock for grafting in melons, and the breeding of cadmium-resistance rootstocks plays a key role in the safe production of melon vegetables. However, to our knowledge, this is the first report of research on cadmium-tolerant specific rootstocks.

In this study, the cadmium-tolerant rootstock resource previously screened included China pumpkin inbred lines 360-3 and 041 – 1, as well as their cross combination (360-3×041 – 1), were used as experimental materials. The effects of cadmium stress on their growth, development, physiological property and cadmium accumulation characteristics were studied, and the defense responses of 360-3×041 – 1 and its parents, including those of 360-3 and 041 – 1 to cadmium, were discussed. This provided a theoretical foundation for the breeding of cadmium-tolerant rootstock for melon vegetables.

Experimental Materials And Methods

Experimental Materials and Experimental Design

China pumpkin inbred lines 360-3 and 041-1, as well as their cross combination (360-3×041-1) used in this study were provided by the Pumpkin Research Group in the College of Horticulture and Gardening, Henan Institute of Science and Technology, Xinxiang, China.

The experiment was conducted in the cultivation laboratory in the College of Horticulture and Gardening at the Henan Institute of Science and Technology between June 25 and August 30, 2020.The substrate culture was applied with a pot bottom diameter of 8.5cm, a pot opening diameter of 12cm and a depth of 10.8cm. The substrate utilized a 3:1:1 ratio of peat, vermiculite and perlite. One kilogram of three-nutrient compound fertilizer was added per cubic substrate, and 0.2kg carbendazim was added per cubic substrate for sterilization. Thus, the water content in substrate reached as high as 70%.

Pumpkin seeds with large grains and an even size were selected and soaked for 7 minutes in warm water that was between 55ºC and 60°C. After cooling to room temperature, they were soaked in distilled water for 6-8 hours. Germination was accelerated in an incubator with a temperature of 25~28°C. After the white bud became exposed, the seeds were sown in one plant per pot. When two cotyledons of seedlings were expanded, a solution without $Cd^{2+}$was used as the control, while cadmium sulfate solutions with Cd^{2+}mass concentration of 8, 16 and 24mg·L^{-1}wereapplied on July 9, 12, 15, 20 and 23. The dosage for each plant was 10mL, which was watered near the plant root system using a pipette, and the various indices were determined on August 10.

The Determination Method of Test Items

The determination of plant height, stem diameter and biomass

Three plants were randomly selected in each treatment and used to determine the plant height and stem diameter. A tape was used to measure the aboveground height (from the plant base to growing point), and a Vernier caliper was used to measure the stem diameter (from the stem base to the half part of two cotyledons). The seedlings were washed with tap water and then three times with distilled water. The plants were divided into the aboveground and below ground parts (all parts contained root hairs) using scissors, dried for 30 minutes at105°Cto remove the water and then dried at 70°C until a constant weight was reached. The dry weight was then measured.

Measurement of relative conductivity, the activities of superoxide dismutase, POD and catalase, as well as the content of malondialdehyde in pumpkin seedling leaves

The measurement on relative conductivity was conducted as described by Dresler et al.(2014). The activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), as well as the content of malondialdehyde (MDA) were measured as described by Li et al.(2000), which their concentrations had increased slightly.

Measurement ofthe morphological indices and activity of root system

The morphological indices of root system were measured as follows: Three seedlings were randomly selected in each treatment, and their root systems were washed and cleaned with tap water. The roots were placed on root plate of a root scanner (EPSON perfection 4990 PHOTO, EPSON Co., Ltd., Beijing, China) for scanning. After processing the photos of scanned root system, various parameters, including the total root length, total projected area, root surface area, the average diameter of root system, total root volume and root tip number, were obtained through an analysis using professional root analytics software (Win RHIZO Pro 2007, Regent Instruments, Quebec City, Canada) on March 13, 2007.

The TTC method was applied to measure the activity of root system as previously described (Li H S.2000).

Measurement ofthe photosynthetic characteristics of pumpkin seedling leaves

A LI-6400 portable photosynthesis measurer (LI-COR, Lincoln, NE, USA) was utilized tomeasurethe photosynthetic characteristics of pumpkin seedling leaves.

Measurement of the cadmium contents in each organ of pumpkin seedling.

Three pumpkin seedlings were randomly selected in each treatment. Dried samples of approximately 0.2g of the stems, leaves and root systems were weighed and ground in a mortar. A volume of 7ml of concentrated nitric acid and 2ml of hydrogen peroxide were added in a digestion tank and placed in a digestion instrument at 165°Cat 5w for 30 – 60 minutes until the solution was clarified, and no impurities remained. The digested solution was transferred to a 50ml polytetrachloro ethylene beaker, and the acid was removed on a 170ºC hot plate. The chlorine was removed until it was nearly dry, and 0.5% nitric acid was used to dilute the sample to 10ml in a centrifuge tube. The mass concentration of cadmium in the samples was measured using an Optima 2100 DV inductively coupled plasma atomic emission spectrometer (ICP-AES) (Perkin Elmer, Waltham, MA, USA)(Chen et al. 2012b).

Transfer coefficient % = heavy metal content in different part/heavy mental content in root system * 100 (Wassenaar et al. 2020)

Isolation and analysis of the subcellular components in pumpkin seedling root system

The method of Song et al.(Song et al. 2011) was utilized and slightly improved. In each treatment, the root systems of three seedlings were randomly selected, cut into pieces and mixed. A total of 0.3g fresh samples were taken and ground in a mortar at 4ºC. A volume of 5mL of homogenate was added, and the components consisted of 250 mmol·L^{-1} sucrose, 50 mmol·L^{-1} Tris-HCl (pH 7.5) and 1 mmol·L^{-1} DTT), transferred into a centrifuge tube and centrifuged at 3 000 r·min^{-1} for 1 minute. The precipitate comprised the cell wall components. The supernatant was taken and centrifuged at 14500 r·min^{-1} for 45 minutes, and the precipitate was organelles, while the supernatant was cytoplasm. The centrifuged cell wall and organelle components were digested again. Diluted nitric acid with a mass ratio of 0.5% was used to dilute the sample to 10mL, and the cytoplasm components were directly diluted in avolumetric flask for measurement.

Statistical analysis

The experimental results were analyzed used Data Processing system 7.55 software (DPS7.55) and Microsoft Excel 2007 (Redmond, WA, USA), and a Duncan analysis was applied for difference analysis. All experiments were performed and analyzed separately with at least three biological replicates.

Results

The effects of cadmium stress to the growth and development of pumpkin cadmium-tolerant rootstock resource and cross combination

The plant seedlings had a sensitive response to cadmium stress, and the phenomena included a physiological metabolic disorder, as well as slowed growth and development (Wang et al. 2020). Table 1 shows that with the increased mass concentration of cadmium stress, the growth of 360-3×041 − 1 and its parents were suppressed to different levels. Only under conditions without the addition of cadmium, was the plant height of 360-3×041-1significantly lower than that of the male parent. With the increase in mass concentration of cadmium stress, there was a non-significant difference on the seedling growth compared with those of the parents.

Table 1
The effects of cadmium stress on the plant height and stem diameter of pumpkin seedlings.

concentration of cadmium stress(mg·L^{-1})	Plant height(cm)			Stem thickness(mm)		
	360-3	041 – 1	360-3×041 – 1	360-3	041 – 1	360-3×041 – 1
ck	10.83 ± 0.74b	13.60 ± 0.73a	10.27 ± 0.65b	5.07 ± 0.46a	4.94 ± 0.41a	4.80 ± 0.05a
8	10.57 ± 1.52a	10.97 ± 0.33a	11.97 ± 1.05a	3.66 ± 0.34b	4.40 ± 0.27a	4.35 ± 0.24ab
16	11.50 ± 0.82a	12.17 ± 0.97a	12.50 ± 0.82a	4.21 ± 0.20a	3.94 ± 0.51a	4.08 ± 0.38a
24	10.80 ± 0.64a	12.03 ± 0.70a	11.70 ± 0.91a	4.20 ± 0.18a	3.87 ± 0.74a	3.96 ± 0.30a

Note: Different lowercase letters indicate significant differences between different materials under the same stress Concentration ($P < 0.05$), the same below.

Table 2 shows that under the cadmium stress with a higher mass concentration of 24mg·L^{-1}, the biomass of aboveground parts of 360-3×041-1increased by 5.28%~11.44% compared with those of the parents, while the biomass of belowground parts increased by 37.88%~15.19% compared with those of the parents.

Table 2
The effects of cadmium stress on the biomass of pumpkin seedlings.

concentration of cadmium stress(mg·L^{-1})	Aboveground shoot dry weight(g)			Underground root dry weight(g)		
	360-3	041 – 1	360-3×041 – 1	360-3	041 – 1	360-3×041 – 1
ck	1.349 ± 0.07ab	1.404 ± 0.26a	0.953 ± 0.10b	0.110 ± 0.03a	0.096 ± 0.04a	0.060 ± 0.01a
8	0.556 ± 0.13a	1.369 ± 0.05b	1.324 ± 0.05b	0.039 ± 0.01a	0.119 ± 0.01a	0.117 ± 0.06a
16	1.182 ± 0.07a	1.240 ± 0.13a	1.112 ± 0.22a	0.084 ± 0.01b	0.112 ± 0.01a	0.109 ± 0.01a
24	1.268 ± 0.19a	1.198 ± 0.06a	1.335 ± 0.11a	0.079 ± 0.01a	0.066 ± 0.01a	0.091 ± 0.01a

The effects of cadmium stress on membrane lipid peroxidation and membrane protective enzyme activity in pumpkin cadmium-tolerant rootstock resource and cross combination

Figure 1 shows that the conductivity of 360-3×041 – 1 and its parent leaves first increased, decreased and then increased again with the increase in mass concentration of cadmium stress. Under the same concentration of cadmium stress, there were significant differences between various varieties. When the mass concentration of cadmium stress reached its maximum, the relative conductivity of 360-3×041 – 1 was significantly lower than those of the parents, which decreased by 35.86%~36.31%.

Figure 2 shows that with the increase in concentration of cadmium stress, the content of MDA in seedling leaves of 360-3×041 – 1 and its parents generally increased. This indicated that cadmium stress resulted in damage to the leaves of three materials to some extent. The content of MDA in 360-3×041-1was only significantly higher than that of the female parent under conditions without cadmium stress, while under the cadmium stress with mass concentration of 8 ~ 24mg·L^{-1}, there was a non-significant difference in the content of MDA between 360-3×041 – 1 and its parents.

As membrane protective enzymes, SOD, POD and CAT can oxidize and decompose the reactive oxygen species (ROS) into non-toxic water and oxygen, thus, reducing the toxicity of heavy metals to plants(Zhao et al. 2019). Figure 3 shows that under the cadmium stress with a higher mass concentration of 24mg·L^{-1}, SOD activity of 360-3×041 – 1 was significantly higher than that in the male parent 041 – 1 (Fig. 3A). Under the cadmium stress with mass concentrations of8mg·L^{-1} and 16mg·L^{-1}, the POD activity of 360-3×041 – 1 was higher than those of the parents, whereas at the concentration of 16mg·L^{-1}, it differed significantly compared with the male parent 041 – 1 (Fig. 3B). Under the cadmium stress with different mass concentrations, CAT activity of 360-3×041 – 1 was always between that of the parents, which had not reached significant differences (Fig. 3C).

The effects of cadmium stress on the root system growth of pumpkin cadmium-tolerant rootstock resources and cross combination

The plant root system is the organ that suffers first from cadmium toxicity, and the activity of root system is one of the key indices that represents the growth status and activity level of pumpkin seedling root systems (Ba et al. 2017). Figure 4 shows that under cadmium stress with different mass concentrations, there were significant differences on the root system activity between 360-3×041 – 1 and the parents. With the increase in mass concentration of cadmium stress, the root systems of 360-3 and 041 – 1 were all damaged to differing levels, and the root system activities also decreased. However, the root system activity of 360-3×041-1increased with the increase in mass concentration of cadmium stress. When the mass concentration of cadmium stress reached 16mg·L^{-1} and 24mg·L^{-1}, the root system activity of 360-3×041 – 1 was significantly higher than those of the parents and increased by 18.80%~22.05% and 89.85 ~ 91.45% compared with those of the parents, respectively.

After scanning with the root system scanner, the root system pictures were used to observe the root hair numbers of 360-3×041 − 1 and its parents under cadmium stress with different concentrations. It was found that with the increase in concentration of cadmium stress, the root hair numbers of 360-3 and 041-1obviously decreased. The root hair numbers of 360-3×041-1decreased under the stress with a concentration of 8mg·L^{-1}, while it increased under the cadmium stress with higher concentrations, and it was higher than those of the parents (Fig. 5).

With the increase in mass concentration of cadmium stress, the total root length, total projected area, root surface area, total root volume and root tip number of 360-3×041 − 1 and its parents decreased, while the average diameter of root system tended to first increase and then decrease(Fig. 6A-F). Under conditions without cadmium stress, the total root length, total projected area, root surface area, total root volume and root tip number of 360-3×041 − 1 were between those of the parents, and the differences were significant (Fig. 6A-F). With the increase in mass concentration of cadmium stress, with the exception that the total root length of 360-3×041 − 1 was lower than those of the parents at a concentration of 8mg·L^{-1} (Fig. 6A), the total root length, total projected area, root surface area, total root volume and root tip number were all higher than those of the parents under cadmium treatment with other mass concentrations. However, the differences were not significant (Fig. 6A-F), and the root system growth showed super-parent heterosis.

The effects of cadmium stress on the photosynthetic characteristics of pumpkin cadmium-tolerant rootstock resources and cross combination

Figure 7 shows that under cadmium stress with different mass concentrations, the net photosynthetic rate of 360-3×041 − 1 and its parents tended to first decrease and then increase, and the net photosynthetic rate of 360-3×041 − 1 was always higher than those of its parents (Fig. 7A). However, the differences were not significant. The stomatal conductance of 360-3×041 − 1 and its parents reached their maximum under cadmium stress with a concentration of 8mg·L^{-1}.With the increased mass concentration of cadmium stress, the stomatal conductance of 360-3×041 − 1 was always higher than those of its parents and significantly higher than that of its male parent 041 − 1 (Fig. 7B). The transpiration rate of 360-3×041 − 1 was always lower than those of its parents, except that under conditions without cadmium stress, it was significantly lower than that of 041 − 1.There were non-significant differences compared with those of the parents under the cadmium treatment with other mass concentrations (Fig. 7C). With the increase in mass concentration of cadmium stress, the intercellular carbon dioxide of 360-3×041 − 1 was higher than those of its parents, whereas it was significantly higher than that of the male parent 041 − 1 at the concentrations of 16mg·L^{-1} and 24mg·L^{-1} (Fig. 7D).

The effects of cadmium stress on cadmium accumulation characteristics and subcellular distribution of pumpkin cadmium-tolerant rootstock resources and cross combination

As shown in Fig. 8, under the cadmium stress with different mass concentrations, the root system of 360-3×041 − 1 and its parents were the part that had the highest accumulation of cadmium, followed by the stems and leaves. The cadmium distribution rule in each organ of 360-3×041 − 1 and 360-3 was root > stem > leaf, while in 041 − 1, it was root > leaf > stem. Compared with the parents, with the exception that the accumulation of cadmium in the root system of 360-3×041 − 1 was lower than those of its parents under stress with a concentration of 8mg·L^{-1}, it was higher than those of both parents under the cadmium treatments at other mass concentrations. Under the cadmium stress with higher mass concentrations, the accumulation in root system of 360-3×041-1increased.The proportions that were distributed in the in stems and leaves were lower than those of its parents. Simultaneously, the accumulation of cadmium in leaves was also reduced.

In Table 3, the subcellular distribution of cadmium mass concentration in pumpkin seedling roots under cadmium stress with different mass concentrations was analyzed. Table 3 shows that there were significant differences in the subcellular distribution of the mass concentration of cadmium in the root systems between different materials. In 360-3×041 − 1 and its parents, most cadmium accumulated in the cell walls, followed by the cytoplasm and organelles. In contrast, the subcellular distribution rule of cadmium mass concentration in the root system of 360-3×041 − 1 and 041 − 1 seedlings was cell wall > cytoplasm > organelle, while it in the 360-3 seedlings, it was cell wall > organelle > cytoplasm.

Table 3
The effects of cadmium stress on the subcellular distribution of cadmium mass concentration in pumpkin seedling roots.

concentration of cadmium stress(mg·L^{-1})	**cell wall**			**cytoplasm**			**organelle**		
	360-3	**041 − 1**	**360-3×041 − 1**	**360-3**	**041 − 1**	**360-3×041 − 1**	**360-3**	**041 − 1**	**360-3×041 − 1**
ck	0.61 ± 0.21a	0.42 ± 0.17a	0.44 ± 0.15a	0.66 ± 0.15a	0.37 ± 0.28b	0.30 ± 0.16b	0.94 ± 0.15a	0.32 ± 0.17b	0.29 ± 0.22b
8	2.33 ± 0.43a	0.93 ± 0.11b	0.72 ± 0.11b	0.98 ± 0.14a	0.57 ± 0.32b	0.44 ± 0.16b	1.80 ± 0.23a	0.52 ± 0.27b	0.18 ± 0.11c
16	2.92 ± 0.25a	1.01 ± 0.29c	2.21 ± 0.59b	2.32 ± 0.21a	0.34 ± 0.22b	0.52 ± 0.27b	2.06 ± 0.35a	0.55 ± 0.14b	0.30 ± 0.08b
24	2.89 ± 0.11a	1.01 ± 0.22c	2.44 ± 0.35b	0.57 ± 0.18ab	0.42 ± 0.10b	0.80 ± 0.21a	1.98 ± 0.27a	0.26 ± 0.19b	0.27 ± 0.20b

The effects of cadmium stress on cadmium transfer coefficient of pumpkin cadmium-tolerant rootstock resource and cross combination

Figure 9 shows that with the increase in mass concentration of cadmium stress, the transfer coefficient of 360-3 tended to decrease first and then increase, while both041-1 and 360-3×041-1exhibited a tendency to decrease. In addition, the decrease in 360-3×041 − 1 was more significant, whereas it was obviously lower than 360-3 with the maximum mass concentration of cadmium stress. Under the lower mass concentration of cadmium stress, the transfer capacities to the aboveground parts in 041 – 1 and 360-3×041 – 1 were much higher, while both decreased under a higher mass concentration of cadmium stress. The cadmium transfer capacity in 360-3×041 – 1 was significantly lower than those of its parents. Most cadmium was fixed in the roots, which limited its transport to the aboveground parts.

Discussion

Cd in the soil can enter into a plant through absorption by the roots, which generates toxicity to plants and thus, affects their growth. There are some differences on the cadmium tolerance in different varieties even in the different tissues of the same plant, and the growth of more tolerant plants was less affected by toxicity (Zhao et al. 2015). Previous research (Xiang et al. 2020; GAO et al. 2020; Zhang et al. 2013) indicated that cadmium stress had different degrees of suppression on seed germination, seedling growth (plant height and stem diameter) and dry weight. In this study, with the increase in mass concentration of cadmium stress, the plant height and stem diameter of 360-3, 041 – 1 and 360-3×041 – 1 were suppressed, but no significant effects occurred. In addition, no significant cadmium toxicity symptom appeared during growth. Under cadmium stress with a higher mass concentration of 24mg·L^{-1}, the biomass of aboveground parts of 360-3×041-1increased by 5.28%~11.44% compared with those of the parents, while the biomass of belowground parts increased by 37.88%~15.19% compared with those of the parents. However, the differences were not significant. This indicated that all three materials had a stronger tolerance to cadmium, and there was a non-significant difference on the growth between different materials, which might be related to parents that also had a higher tolerance to cadmium.

The heavy metal stress with a high mass concentration will lead to the generation of abundant reactive oxygen species (ROS) in the plant cells, which will result in membrane lipid peroxidation caused by unsaturated acids in the plasma membrane (PM)and thus, increased its permeability(Chen et al. 2014; Lei et al. 2018) The cell PM is the main part of the plant that is damaged by stress. Generally, the relative conductivity, namely the permeability of PM, is used to reflect the extent of plant injury under stresses (Xia et al. 2009). As the final product of membrane lipid peroxidation, MDA is an important index that reflects its effects. It can cause damage to proteins by generating covalent complexes, which could be involved in the damage to tissues during aging (Traverso et al. 2004; Hodges et al. 2009). Zhao(2015)found that with the increase in mass concentration of cadmium stress, as well as the extension of its duration, the membrane permeability of corn leaves was increased, which resulted in an increase in the relative conductivity in leaves and thus, affected the growth and development of corn. Heavy metal stress also leads to an increase in the content of MDA in many plants, which caused the imbalance of system to generate and eliminate the ROS in plants(Khatun et al. 2008; Gajewska et al. 2008). SOD, CAT and POD are the important membrane protective enzymes to eliminate the reactive oxygen in plants under stress.SOD can effectively eliminate O^{2-} in plants and transform it into $H_2O_2$with a weaker oxidizing capacity, and then it will be decomposed into H_2O and O_2 through the activities of POD (Zhao et al. 2015). Thus, this process will reduce the damage of membrane lipid peroxidation. In this study, with the increase in mass concentration of cadmium stress, the conductivity in leaves of 360-3×041－1 and its parents all tended to first increase, then decrease and increase again. Under the cadmium stress with the highest mass concentration of 24 mg·L^{-1}, the relative conductivity in 360-3×041－1 was significantly lower than those of its parents. Under cadmium stress with different mass concentrations, the content of MDA tended to increase. Under cadmium stress with amass concentration of 8 ~ 24mg·L^{-1}, there were non-significant differences in the content of MDA between 360-3×041－1 and its parents. With the increase in mass concentration of cadmium stress, the activity of SOD tended to first decrease and then increase. Under cadmium stress with a mass concentration of 24mg·L^{-1}, the activity of SOD in 360-3×041－1 was significantly higher than that of the male parent, while it had a non-significant difference compared with the female parent. At the concentration of 8mg·L^{-1} and 16mg·L^{-1}, the activity of POD in 360-3×041－1 was higher than those of its parents, while it had a significant difference compared with that of the male parent at 16mg·L^{-1}. Under cadmium stress with different mass concentration, the activity of CAT in 360-3×041－1 had non-significant differences compared with its parents. The increase in SOD activity initiates and enhances the protective capability of the PM, which can transform highly toxic ROS to H_2O_2, which has a weaker oxidizing capacity, and further stimulate the increase in activity of POD(He et al. 2015). This indicated that the capacity to eliminate oxygen radicals in 360-3×041－1 was higher than those of its parents, where as POD played a key role in relieving cadmium stress, which was consistent with the results of Wan et al.(2015).

The effects of soil stresses to root system are the most direct. The disruption in growth of the root system will directly affect the supply of nutrients and water to the aboveground parts of plants. The root system growth has plasticity, and it can adapt to stresses by changing the root configuration parameters under heavy metal stresses (JIA et al. 2008). Previous research (Srinivasarao et al. 2004; Chen et al. 2014; Mauchamp et al. 2001; He et al. 2015; Lin et al. 2001) indicated that under stresses, the growth of plant roots was suppressed; the root activity was decreased, and the changes in root system included the inhibitory effects on total root length, total root surface area, root volume and root tip number. In this experiment, under cadmium stress with different mass concentrations, the root growths of 360-3, 041－1 and 360-3×041－1 were suppressed to differing extents. With the increase in mass concentration of cadmium stress, the root activities of parents 360-3 and 041-1gradually decreased, while that of 360-3×041-1tended to increase, which was significantly higher than those of its parents under cadmium stress at higher mass concentrations. Through the observation of root hair numbers and analysis of root morphological indices in 360-3×041－1 and its parents, this indicated that with the increase in degree of cadmium stress, the root hair numbers in 360-3 and 041-1decreasedsignificantly.The root hair numbers in 360-3×041-1increased under cadmium stress with a higher concentration, and it was greater than those of the parents. The total root length, total projected area, root surface area and root tip numbers in 360-3×041－1 seedlings were all higher than its parents. This indicated that under cadmium stress, the growth of pumpkin seedling roots was suppressed. The degree of inhibition in 360-3×041－1 was lower than those of its parents 360-3 and 041－1, and the growth of root system presented super parent heterosis.

Photosynthesis has a close relationship with crop growth and yield (Zhou et al. 2015), which renders it one of the important indices to evaluate plant productivity and adapt ability (Suo et al. 2020). The net photosynthetic rate (Pan R Z .2012)is one of the important indices to evaluate photosynthesis and is represented as the accumulation of dry matter in plant leaves. In this study, under the cadmium stress with different mass concentrations, the net photosynthetic rate of 360-3×041-1was always higher than that of its parents and presented super parent heterosis, which indicated that its photosynthetic capacity was higher than that of its parents. The stomatal conductance in 360-3×041 – 1 was higher than its parents under cadmium stress with higher mass concentrations, which had significant differences compared withthe male parent 041-1and increased by 3.37%~24.32% and 6.45%~28.57%, respectively, compared withboth parents. The transpiration rate in 360-3×041 – 1 was always lower than those of its parents, and the differences compared with the parents were not significant. In summary, under the growth conditions with cadmium stress, the photosynthetic metabolic capacity of 360-3×041 – 1 presented super parent heterosis, which was consistent with the results of Zhao et al. (Zhao et al. 2020).

Liu et al.(2017)indicated that Chinese Pennisetum with strong cadmium tolerance had characteristics, such as a developed root system, whereas the root is the key organ for cadmium accumulation. With the increase in cadmium mass concentration, the absorption of cadmium in root system was significantly higher than that in the aboveground parts. However, the cell wall of subcellular components in root system plays a key role in the inhibition of transport of cadmium from the roots to stems (Xue et al. 2014). In this experiment, under cadmium stress with different mass concentrations, the distribution rule of cadmium in the 360-3 and 360-3×041 – 1 seedlings was root > stem > leaf, while in the041-1 seedlings, it was root > leaf > stem. The subcellular distribution rule of cadmium mass concentration in 360-3×041 – 1 and 041 – 1 seedling roots was cell wall > cytoplasm > organelle, while in the 360-3 seedling roots, it was cell wall > organelle > cytoplasm. This indicated that the root system was the main organ for cadmium accumulation in 360-3×041 – 1 and its parents, and most was absorbed by cell walls in the root system.

Long et al.(2014)found that the difference in content of cadmium in rice grains was related to the root absorption and transfer to aboveground parts. Therefore, the screening of rice varieties with cadmium tolerance focus more intensively on the transfer coefficient of cadmium in grains, and the transfer capacity was decided by the rice genotypes. Previous research(Liu et al. 2017; Zhou et al. 2019; Gao et al. 2019; Zhang et al. 2015;) indicated that the plant root system with a transfer coefficient of less than 1 was the main organ for cadmium accumulation. The poorer the transfer capacity of cadmium from the root system to aboveground parts, the lighter the cadmium toxicity in the aboveground parts, and the stronger the ability to repair the damage from soil cadmium pollution. Under cadmium stress with different mass concentrations, the cadmium transfer coefficient of 360-3tended to first decrease and then increase, while those of 041 – 1 and 360-3×041-1tended to decrease with the increase in mass concentrations of cadmium stress. Under stress with higher mass concentrations, the transfer coefficient of 360-3×041-1decreased by 31.57%~65.33% compared with its parents, and it had a significant difference compared with that of the male parent041-1. This indicated that under cadmium stress with a certain mass concentrations, the cadmium transfer capacity of 360-3×041 – 1 to the aboveground parts was lower than those of its parents. Most were fixed to the roots, which could be owing to the massive accumulation of cadmium in the root cell walls, thus, reducing the toxicity to other organelles and its transfer.

Conclusions

The cross combination (360-3×041 – 1) had a higher defense capability compared withits parents under cadmium stress with a certain mass concentrations.Cadmium accumulated in the roots andto a high extent in the cell walls of root system. The transfer capability to aboveground parts was lower than those of its parents, and the cadmium toxicity to aboveground parts was lower than those of its parents. The net photosynthetic rate was always higher than its parents and presented super parent heterosis. The capacity of eliminating reactive oxygen species in 360-3×041 – 1 was higher than those of its parents, whereas the activity of POD played a keyrole in relieving the stress owing to cadmium.

Declarations

Author Contributions:

Bi-Hua Chen and Huai-Xia Zhang designed the experiments, Wei-Li Guo, Jun-Guo Zhou and Xin-Zheng Li analyzed the data, Bi-Hua Chen wrote the manuscript. Bi-Hua Chen and Huai-Xia Zhang performed the experiments, Wei-Li Guo revised the manuscript. Jun-Guo Zhou and Xin-Zheng Li provided the reagents/materials/analysis tools. All authors have read and approved the final manuscript.

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Data availability: All data generated or analyzed during this study were included in this manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: All authors declared that they had no known competing financial interests or personal relationships that seemed to affect the work reported in this article. All authors followed the ethical responsibilities of this journal.

Consent to participate and publish: All authors participated and approved the final manuscript to be published.

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Figures

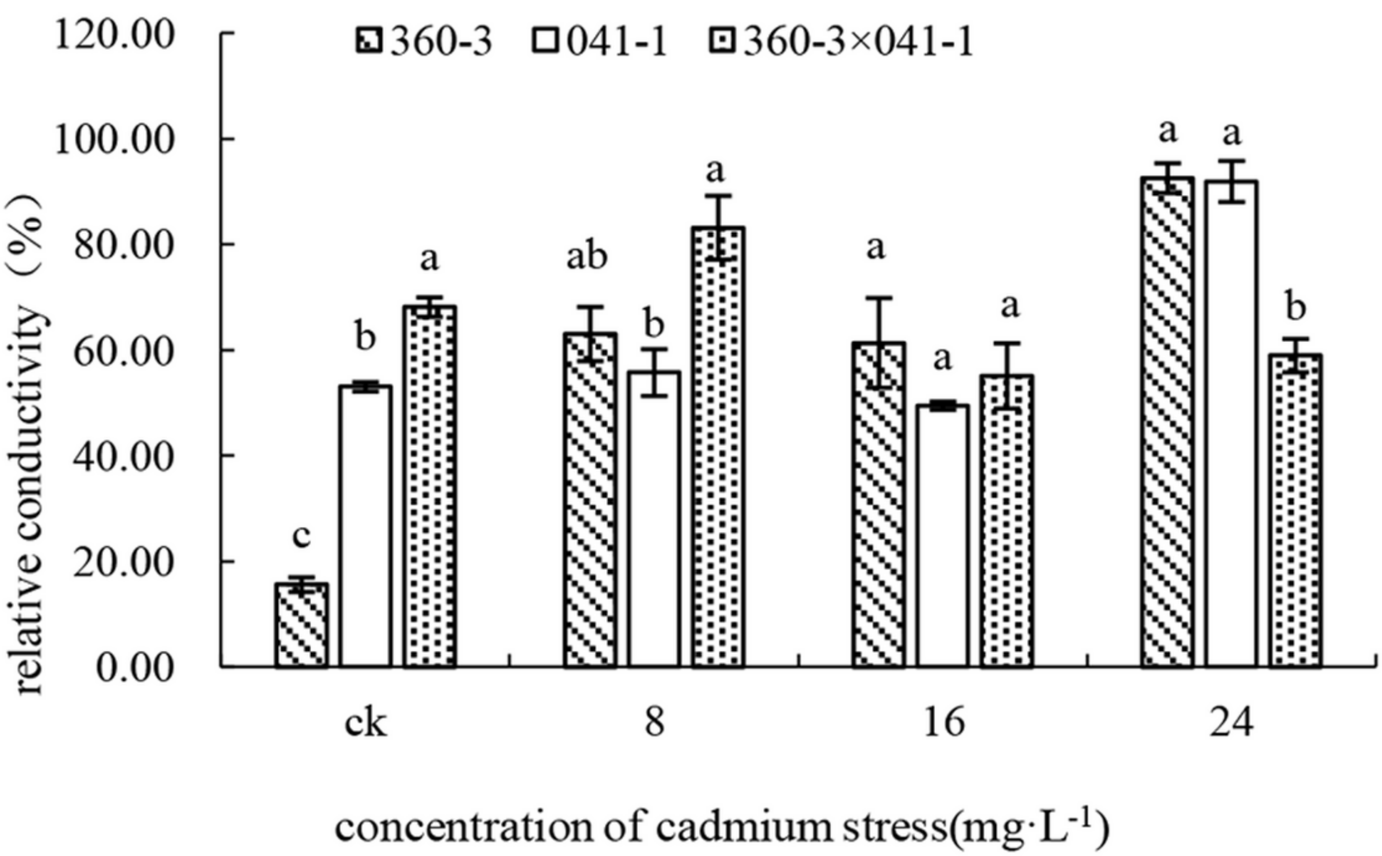

Figure 1

Effect of cadmium stress on relative electrical conductivity of pumpkin seedling leaves. Bars with different letters indicate significant differences at p 0.05. Mean values and SDs for three replicates are shown.

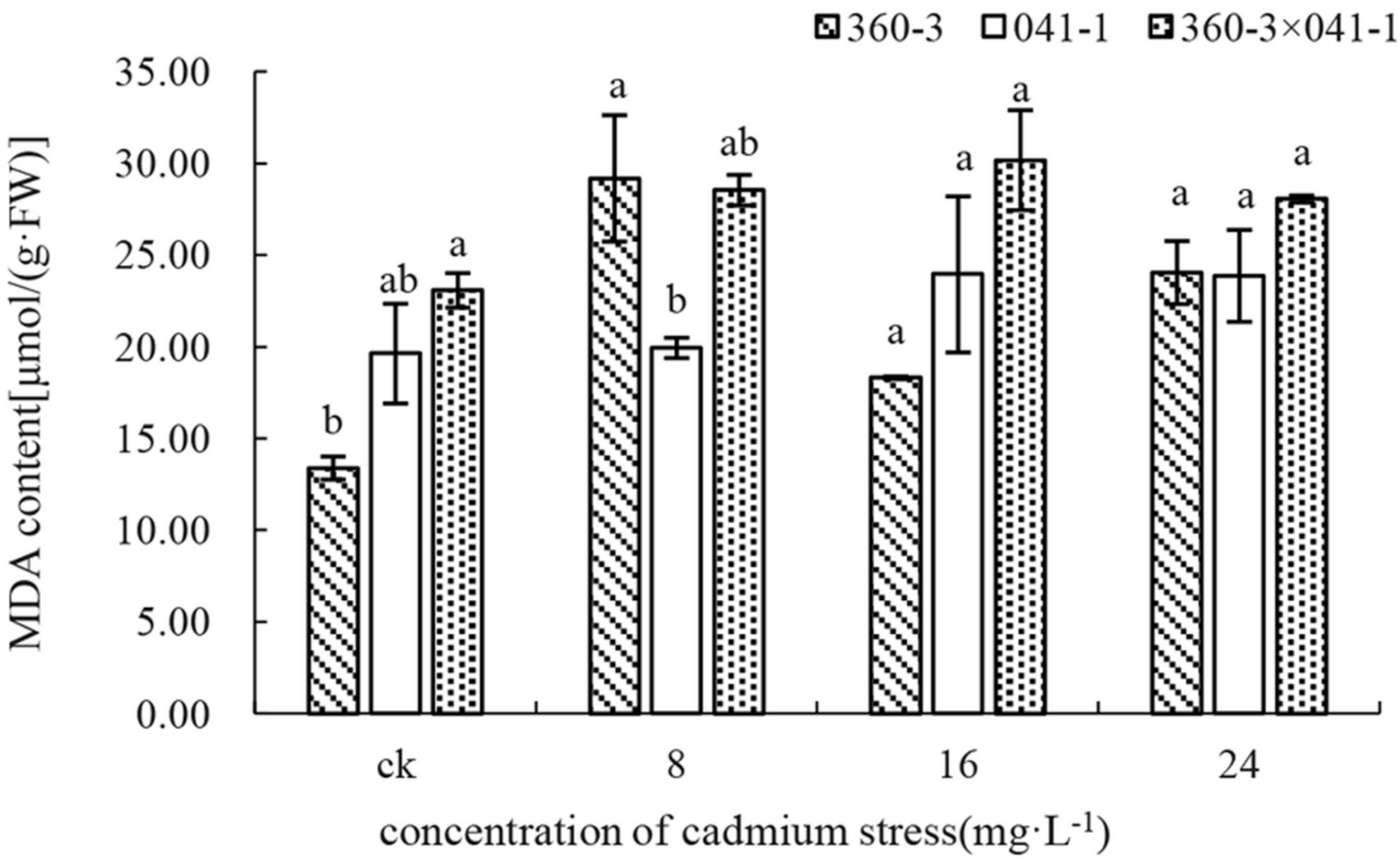

Figure 2

The effects of cadmium stress on the content of MDA of pumpkin seedling leaves. Bars with different letters indicate significant differences at p 0.05. Mean values and SDs for three replicates are shown.

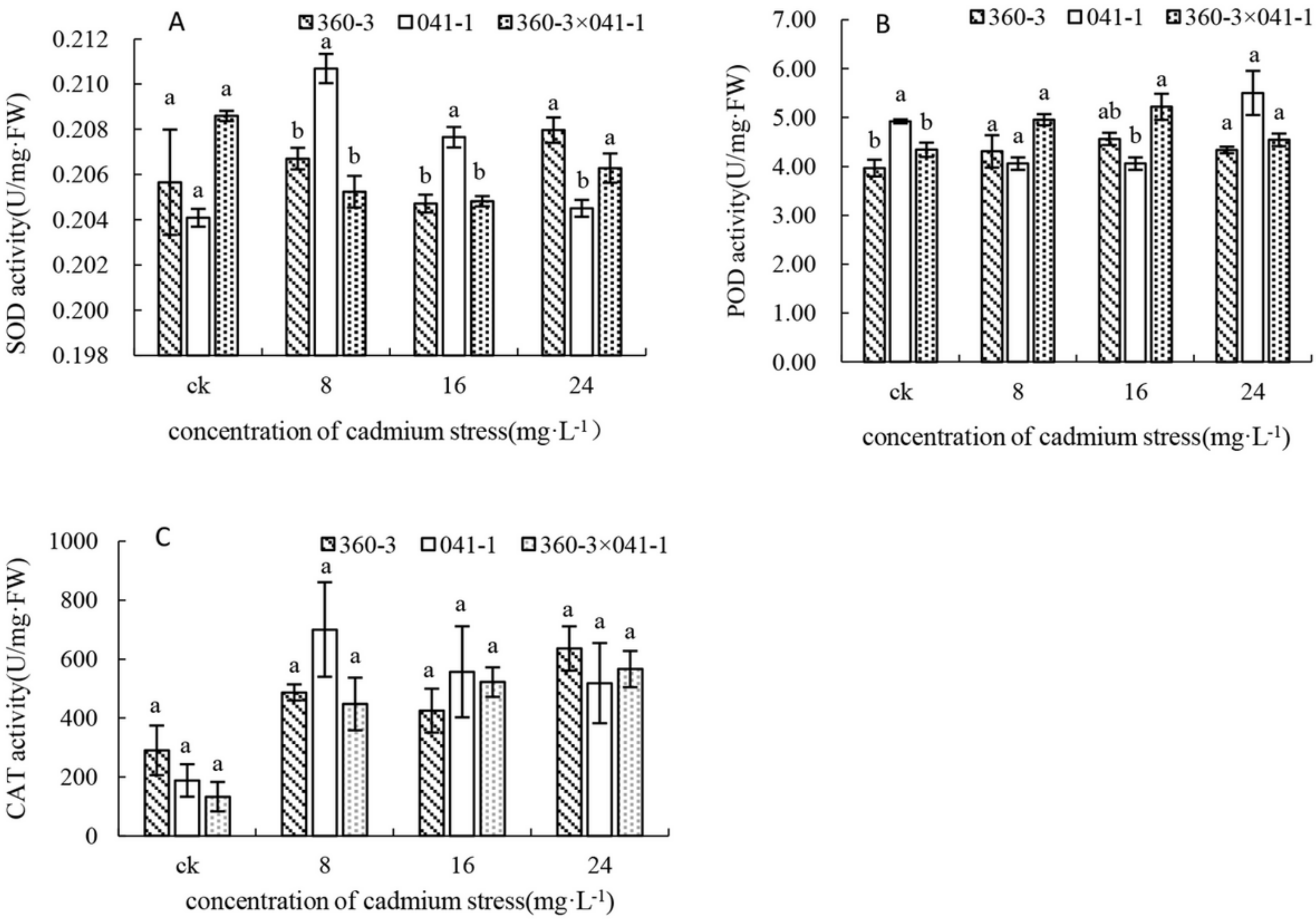

Figure 3

The effects of cadmium stress on the membrane protective enzyme in pumpkin seedling leaves. Bars with different letters indicate significant differences at $p \leq 0.05$. Mean values and SDs for three rep-licates are shown.

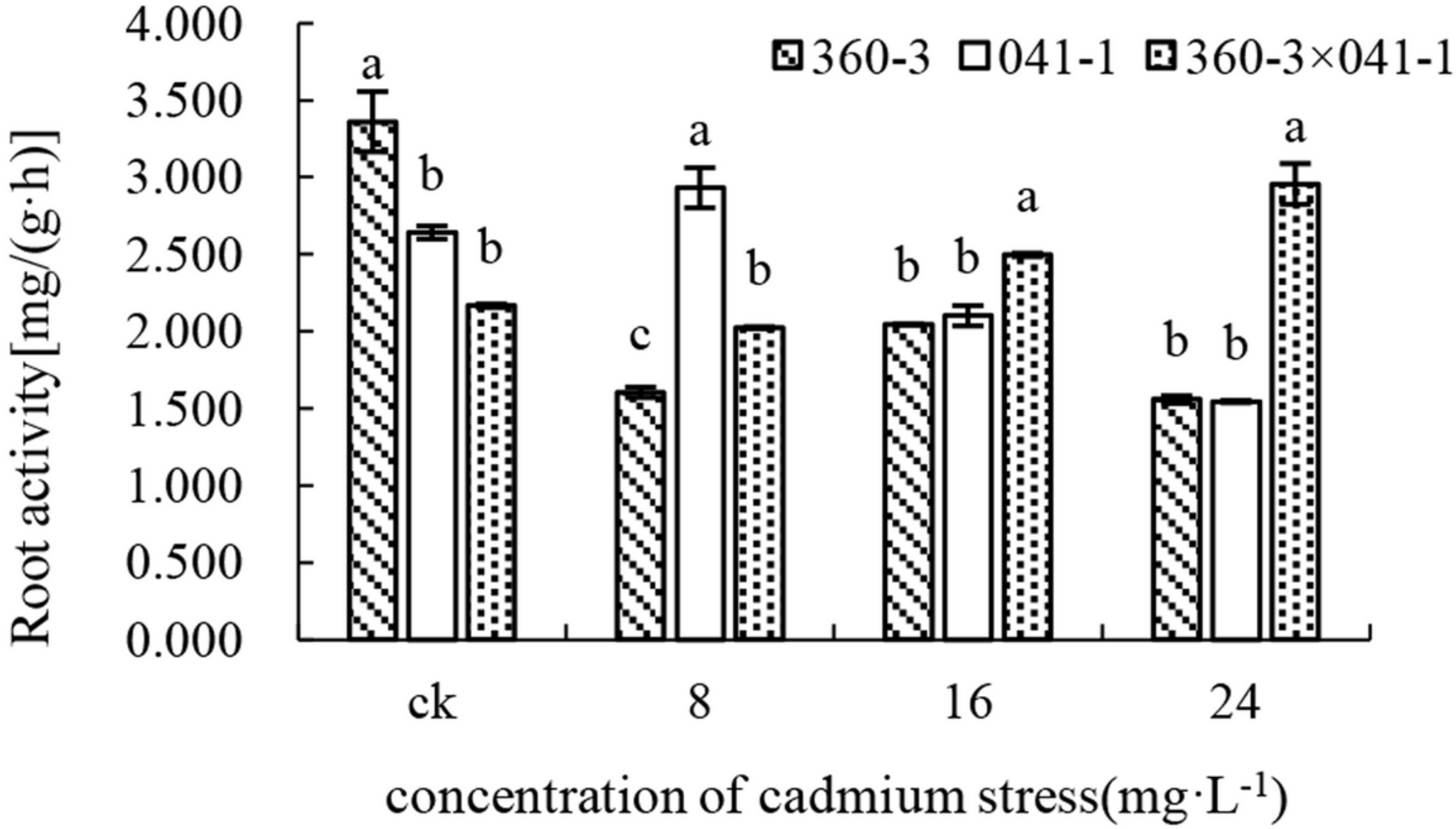

Figure 4

The effects of cadmium stress on root activity of pumpkin seedlings. Bars with different letters indicate significant differences at p 0.05. Mean values and SDs for three replicates are shown.

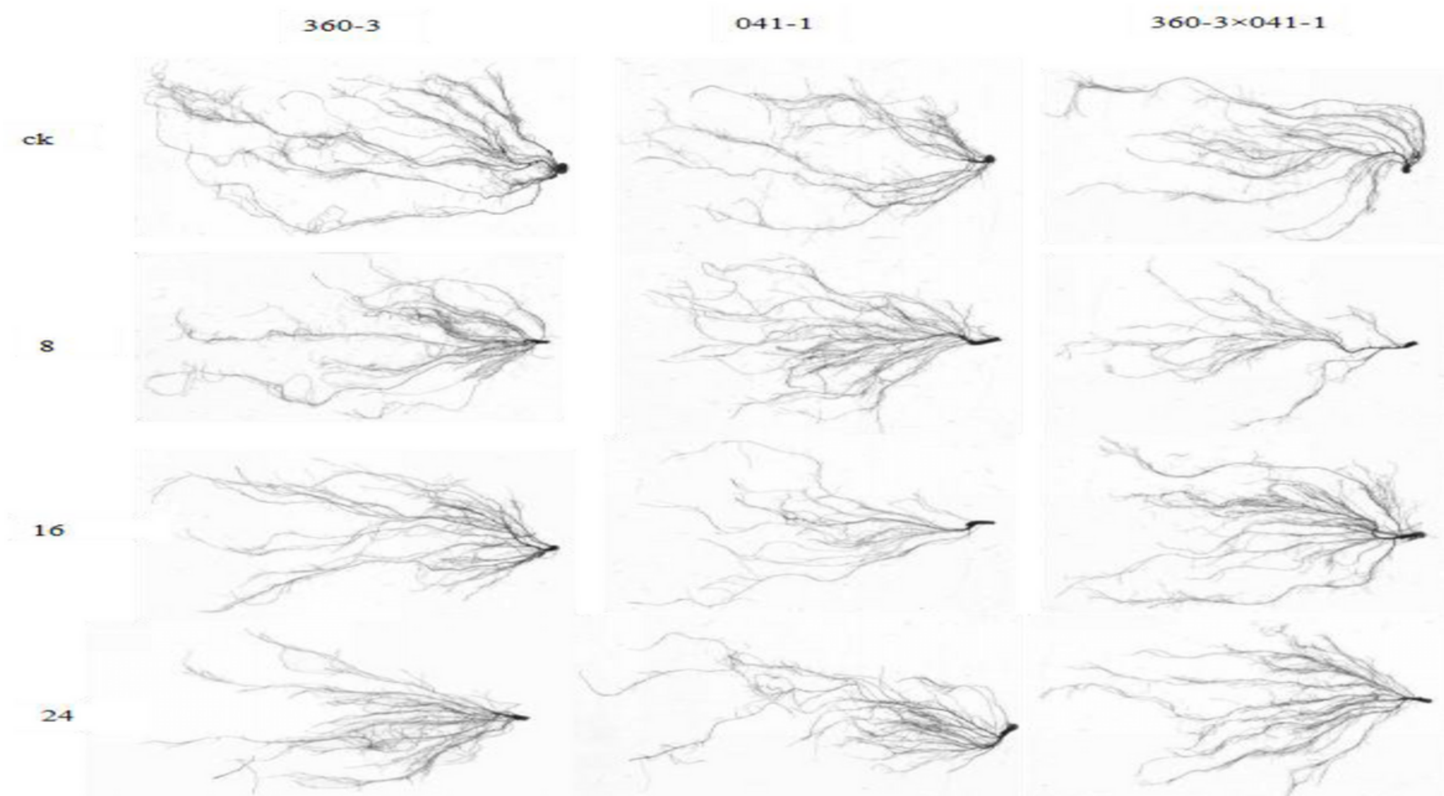

Figure 5

The root system scanning pictures after processing.

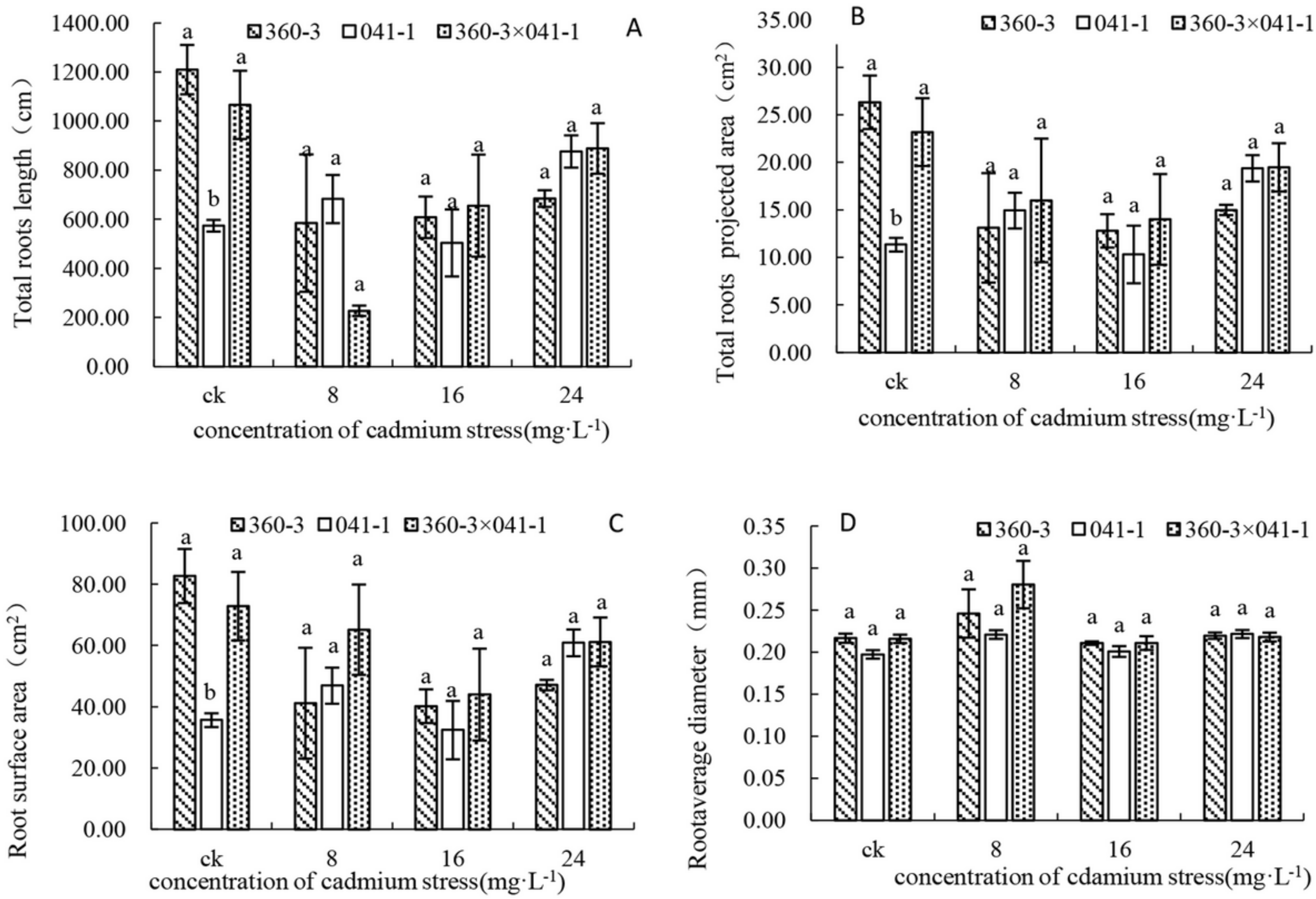

Figure 6

The effects of cadmium stress on the root morphological indexes of pumpkin seedlings. Bars with different letters indicate significant differences at p 0.05. Mean values and SDs for three replicates are shown.

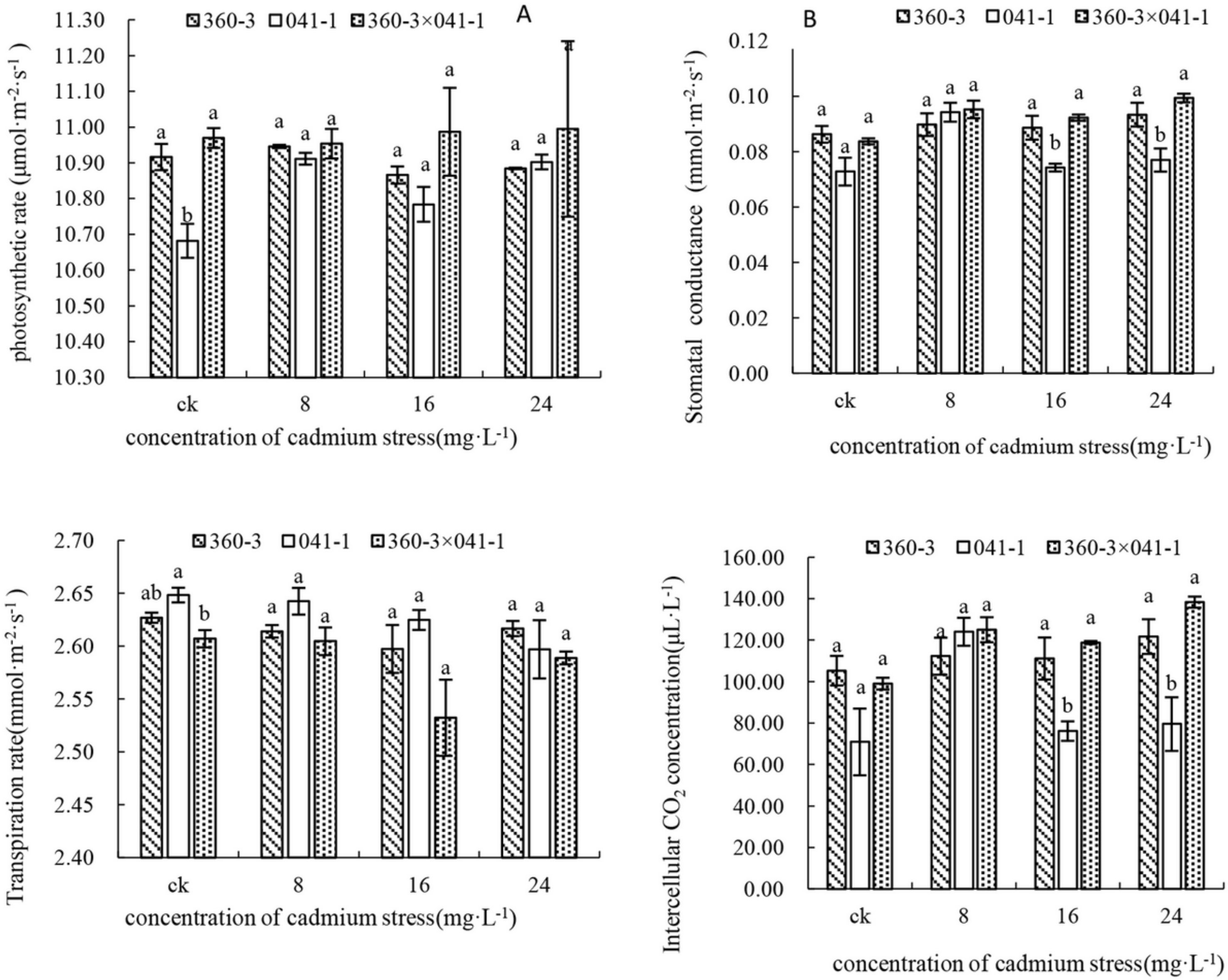

Figure 7

The effects of cadmium stress on photosynthetic characteristics of pumpkin seedlings. Bars with different letters indicate significant differences at p □ 0.05. Mean values and SDs for three replicates are shown.

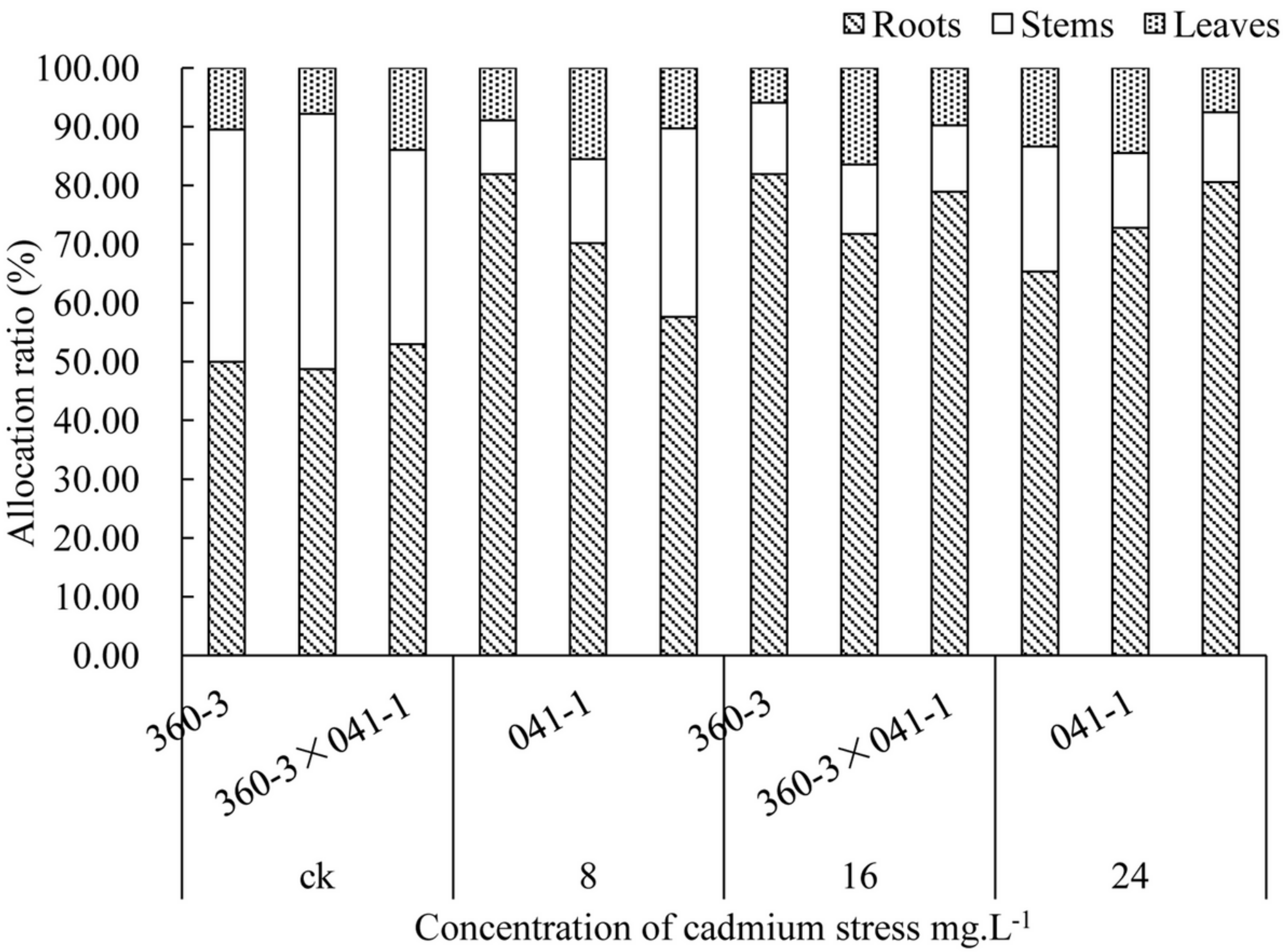

Figure 8

The proportion for mass concentration of cadmium in roots, stems and leaves of pumpkin seedlings under cadmium stress.

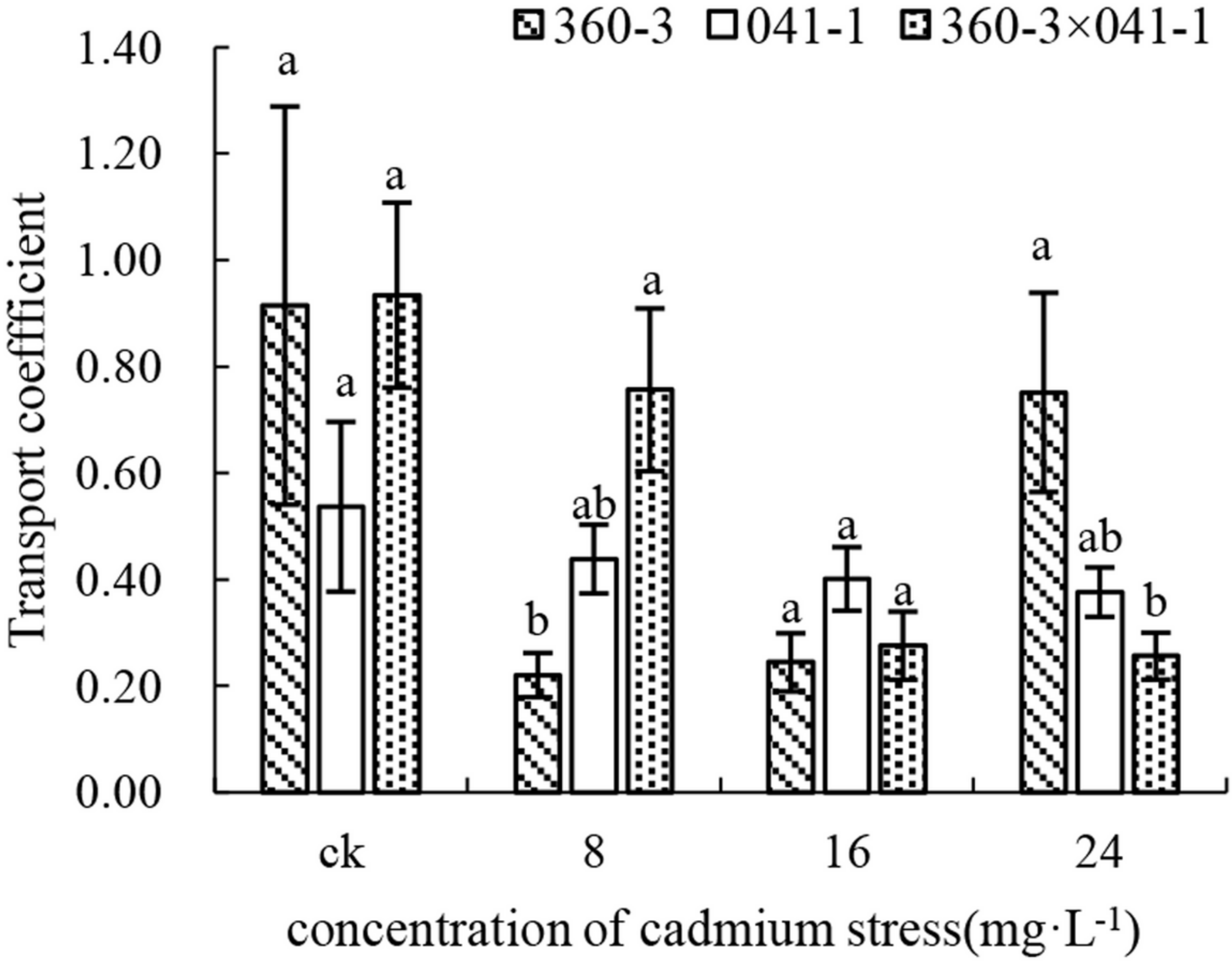

Figure 9

The effects of cadmium stress on cadmium transfer coefficient of pumpkin cadmium-tolerant root-stock resource and cross combination. Bars with different letters indicate significant differences at p 0.05. Mean values and SDs for three replicates are shown.