

The effects of pumpkin rootstock on photosynthesis, fruit mass, and sucrose content of different ploidy watermelon (*Citrullus lanatus*)

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Abstract

Pumpkin rootstock affects watermelon scion growth, fruit yield, and quality, but the mechanisms of related key enzymes and photosynthesis remain unclear. In this study, net photosynthetic rate (P_N), chlorophyll (Chl) fluorescence parameters, fruit mass and sugar accumulation, and related key enzyme activities were determined during the fruit development stage in diploid and triploid watermelon lines and corresponding pumpkin rootstock-grafted lines. The results showed that pumpkin rootstock increased P_N and Chl fluorescence parameters of diploid and triploid watermelon, indicating that pumpkin rootstock could increase photosynthesis, the utilization efficiency of light energy of diploid and triploid watermelon lines. Pumpkin-grafted lines had higher alkaline α -galactosidase activity and lower activities of insoluble acid invertase, sucrose phosphate synthase, and sucrose synthase than corresponding own-root lines in diploid and triploid watermelon. It indicates that pumpkin rootstock could increase the unloading of photoassimilates and reduce the conversion of photoassimilates into sucrose in diploid and triploid watermelon fruits.

Keywords: graft; photoassimilate; quality; triploid; yield.

Introduction

Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] is an important crop because of its sweetness, flavor, high vitamin and nutrient content (Garster 1997, Compton *et al.* 2004). Most watermelon cultivars are diploid and produce fruit that has red flesh with small black seeds at maturity. However, triploid cultivars have been available for over 60 years and are becoming more prevalent (Kihara 1951). Triploid cultivars are preferred by most consumers because of their sweeter taste and lack of hard seeds (Marr and Gast 1991). For this reason, the use of triploid cultivars has increased markedly, and they represent a significant proportion (50%) of the total watermelon production (Maroto *et al.* 2005). Watermelon often suffers from soil-borne pathogens. The lack of resistance to soil-borne pathogens in modern watermelon cultivars is the result of cultivation and selection that have focused on desirable fruit qualities at the expense of disease resistance. A large portion of disease resistance genes has been lost during watermelon domestication (Guo *et al.* 2012, Lin *et al.* 2013). One way to reduce losses in the watermelon performance caused by soil-borne pathogens in plants would be grafting watermelon onto rootstocks with high resistance to soil-borne pathogens, *e.g.*, pumpkin and gourd. To date, grafts have been used to suppress damages

from soil-borne pathogens (Davis *et al.* 2008) and increase the tolerance to temperature change and salt stress, enhance nutrient uptake (Huang *et al.* 2016a), improve water-use efficiency, reduce organic pollutant uptake, limit the toxicity of boron, copper, and cadmium (Colla *et al.* 2010). Grafting of watermelon has developed very quickly in the last 50 years; in the past, pumpkin rootstock grafting was widely used in watermelon to limit the effects of soil-borne pathogens (Albacete *et al.* 2015, Shireen *et al.* 2020). In China, ~20% of the watermelon crop has been grafted to avoid soil-borne diseases, and it has been a routine technique in continuous cropping systems in many countries (Davis *et al.* 2008).

Yield and quality are the main selection traits of crops. Sweetness is a key attribute that determines the fruit quality of watermelon. Sucrose, glucose, and fructose are the main sugars that accumulate in watermelon. Sucrose content increases rapidly during fruit development, while fructose and glucose contents are almost constant during watermelon fruit development (Elmstrom and Davis 1981, Brown and Summers 1985, Kano 1991). Therefore, the sugar content of watermelon fruit is mainly determined by the sucrose content. Recent work showed that multiple factors contribute to the formation of fruit mass and sucrose, including photosynthesis, key enzyme activities relevant to fruit mass accumulation, and sugar

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Abbreviations: Chl – chlorophyll; DAP – days after pollination; ETR – electron transport rate; F_v/F_m – maximum photochemical efficiency of PSII; IAI – insoluble acid invertase; P_N – net photosynthetic rate; SPS – sucrose phosphate synthase; SuSy – sucrose synthase; Φ_{PSII} – actual photochemical efficiency of PSII.

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synthesis (Liu *et al.* 2013, Huang *et al.* 2016a,b). Alkaline α -galactosidase (EC 3.2.1.22), insoluble acid invertase (IAI, EC 3.2.1.26), sucrose synthase (SuSy, EC 2.4.1.13), and sucrose phosphate synthase (SPS, EC 2.4.1.14) are the key enzymes involved in fruit mass accumulation and sugar synthesis in watermelon fruit, to a great extent, determining the fruit yield and sugar content of fruit (Liu *et al.* 2013).

Pumpkin rootstock is reported to increase the fruit mass of watermelon and decrease the sugar content of watermelon fruit (Huang *et al.* 2016a). But until now, no comprehensive data are available on the relationship between pumpkin rootstock and watermelon photosynthesis, key enzyme activities relevant to fruit mass accumulation and sugar synthesis. Therefore, this work aims to investigate how pumpkin rootstock induces changes in fruit mass and sugar content of diploid and triploid watermelon by changing photosynthesis and key enzyme activities relevant to fruit mass accumulation and sugar synthesis.

Materials and methods

Plant materials: In this experiment, a commercial pumpkin rootstock cultivar ‘Baimi112’ (*Cucurbita maxima*, Henan Institute of Science and Technology), commercial triploid watermelon cultivar ‘Zhengzhou No. 3 (3X)’, and corresponding diploid watermelon line ‘Zhengzhou No. 3 (2X)’ [*Citrullus lanatus* (Thunb.) Matsum. and Nakai., Chinese Academy of Agricultural Sciences] were used. The triploid watermelon cultivar ‘Zhengzhou No. 3 (3X)’ was obtained by crossing diploid ‘Zhengzhou No. 3 (2X)’ and corresponding autotetraploid ‘Zhengzhou No. 3 (4X)’ parental lines.

Experimental design: The seeds of pumpkin, diploid and triploid watermelon were surface sterilized with 3% (v/v) sodium hypochlorite for 2 min followed by three washes with sterile deionized water. After germination at 30°C for 24 h, the seeds were sown in 50-cell plug trays filled with a mixture (1:1:1) of peat, perlite, and vermiculite (v/v) in the greenhouse at Henan Institute of Science and Technology, with a 14/10-h day/night photoperiod at temperatures ranging between approximately 22 and 30°C and ambient relative humidity. For grafting, pumpkin rootstock seedlings need to be larger than the scion seedlings. Therefore, the rootstock seeds of pumpkin were sown 5 d earlier than that of the watermelon scion (triploid and diploid watermelon) seeds. Two grafting combinations of watermelon lines were used, *i.e.*, triploid watermelon (3X) grafted onto pumpkin (3X/P) and a diploid watermelon (2X) grafted onto pumpkin (2X/P). Once the pumpkin rootstock seedling produced the second true leaf, grafting was performed by using the hole-insertion grafting method as described by Hassell *et al.* (2008). The ungrafted diploid and triploid watermelon lines were used as control. To maintain high humidity, seedlings were covered with a layer of transparent plastic film, and seedlings were placed in the shade for 72 h. The plastic film was removed for a short time during the initial days to control relative

humidity, and it was completely removed after 10 d from grafting. When the third true leaf emerged, the own-root watermelon and grafted-root watermelon seedlings were transplanted into an open field in Xinxiang, China (35°18'N, 113°52'E), and grown under the same conditions in early May. Each line comprised of two rows and each row included ten individuals. The spacing between the rows was 180 cm, and the spacing between individuals in a row was 50 cm. The treatment was replicated four times and was arranged in a randomized complete block design. The flowers were hand-pollinated and tagged. Five individual fruits were chosen randomly from each line of each treatment at 10, 20, 30 d, respectively, after pollination and used for testing fruit mass, dry matter content (whole fruit), sugar content (flesh), and assaying enzyme activity (flesh). The flesh (central portion) samples were collected and divided into two subsets. One subset was freeze-dried to a powder for sugar content determinations. The other subset was immediately frozen in liquid nitrogen and stored at -80°C for the enzyme assays. Each point, therefore, represents the average of five samples from individual fruit.

The net photosynthetic rate (P_N) was measured under the conditions of the natural environment (field). We also recorded the PFD and temperature at the experimental location at this time (Fig. 1). LI-6400 portable photosynthesis system (LI-COR Co., USA) was used to measure the P_N of the third leaf (from the top) at 10, 20, 30 d, respectively, after pollination under natural conditions. They were measured every hour from 8:00–18:00 h. Each result shown was the mean of ten replicated treatments.

The Chl fluorescence parameters of the third leaf were measured with a portable Chl fluorometer (*Mini-PAM*, Heinz Walz GmbH, Effeltrich, Germany). The mean values of leaf electron transport rate (ETR), maximum photochemical efficiency of PSII (F_v/F_m), and actual photochemical efficiency of PSII (Φ_{PSII}) were measured as described by Baker (2008). Each result shown was the mean of ten replicated treatments.

Sucrose and total sugar determination: Sucrose and total sugar content was assayed as described by Liu *et al.* (2013). Approximately 200 mg of freeze-dried flesh per sample was ground to a fine powder and extracted for 1 h in 10 mL of 50% ethanol at 80°C and then centrifuged at $3,000 \times g$ for 10 min. The pellet was again extracted and centrifuged, and the combined supernatants were placed in a volumetric flask (25 mL); 2 mL of the sample was centrifuged at $3,000 \times g$ for 10 min. The supernatants (1 mL) were filtered through a 0.45- μ m HPLC nylon filter (*Membrana*, Germany). The sugars in the sample were separated in an analytical HPLC system (*Pump System LC-10ATVP*, Shimadzu, Japan) fitted with a *Shodex Asahipak NH2P-504E* column (4.6 \times 250 mm; *Shodex*, Japan) using a refractive index detector (*RID-10A*, Shimadzu, Japan). Each result shown was the mean of five replicated treatments.

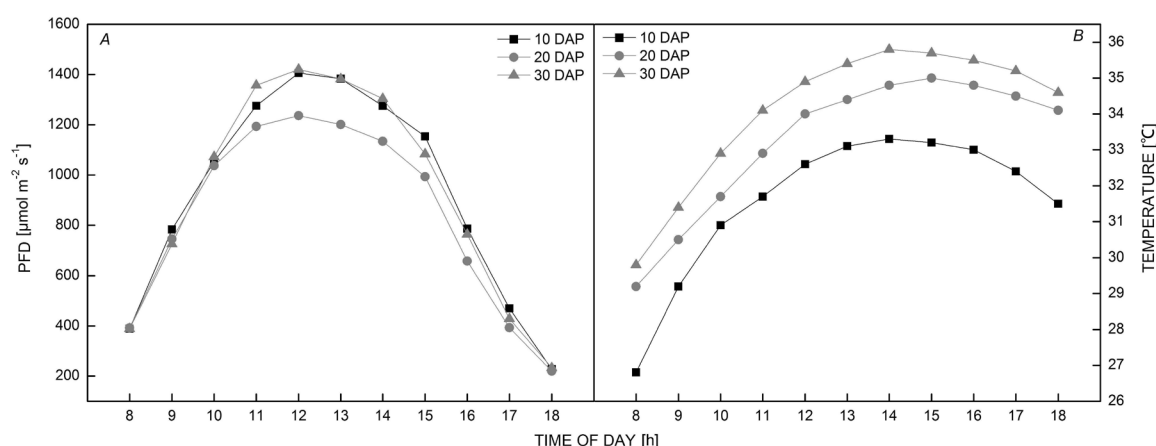


Fig. 1. Diurnal changes of light intensity (A) and temperature (B) at 10, 20, 30 d after pollination. DAP – days after pollination.

Extraction and assay of alkaline α -galactosidase: The alkaline α -galactosidase activity was assayed according to the method of Gao and Schaffer (1999), with some modifications. Approximately 1 g of freshly frozen flesh was homogenized in a mortar with four volumes of extraction buffer containing 50 mM Hepes–NaOH (pH 7.5), 2 mM EDTA, and 5 mM DTT. All samples were centrifuged at $18,000 \times g$ for 20 min at 4°C to separate the supernatants. After centrifugation, the supernatants were collected for alkaline α -galactosidase analysis using *p*-nitrophenyl- α -D-galactopyranoside (pNPG) as a substrate. The initial reaction buffer contained 5 mM pNPG in 50 mM Hepes buffer (pH 7.5). The samples were incubated at 37°C. The reaction was terminated after 10 min by adding four volumes of 0.2 M Na₂CO₃. The release of *p*-nitrophenol was measured spectrophotometrically at 410 nm ($\epsilon = 2.51 \times 10^4$) (S22PC, Shanghai Optical Instrument Factory, China), and *p*-nitrophenol (Sigma) was used as a standard. Each result shown was the mean of three replicated treatments.

Insoluble acid invertase (IAI) extraction and assay: Insoluble acid invertase activity was measured according to the method of Miron and Schaffer (1991), with some modifications. Approximately 1 g of freshly frozen flesh was homogenized in a mortar with three volumes of extraction buffer containing 50 mM Hepes–NaOH (pH 7.5), 0.5 mM Na-EDTA, 2.5 mM DTT, 3 mM diethyldithiocarbamic acid, 0.5% (w/v) BSA, and 1% (w/v) insoluble polyvinylpyrrolidone (PVP). All samples were centrifuged at $18,000 \times g$ for 30 min at 4°C to collect the insoluble pellet (containing crude insoluble acid invertases) by separation. The insoluble pellet was homogenized in the extraction buffer, centrifuged, and suspended in 3 mL of 50 mM Hepes–NaOH (pH 7.5) and 0.5 mM Na-EDTA. To solubilize the ‘insoluble’ acid invertase enzyme, NaCl (0.5 M final concentration) was added to the initial extraction buffer before extraction. All samples were incubated at 4°C for 10 min and centrifuged at $18,000 \times g$ for 20 min at 4°C. The supernatants were collected for analysis of crude insoluble acid invertase activity. Insoluble acid invertase activity was assayed in

0.8 mL of 0.1 M K₂HPO₄–0.1 M citrate buffer (pH 5.0), 0.2 mL 0.1 M sucrose, and 0.2 mL of enzyme extract (for the control). All samples were incubated for 30 min at 37°C, after which the reactions were stopped at 100°C for 5 min. The content of reducing sugar produced during the reactions of insoluble acid invertase was determined using a 3,5-dinitrosalicylic acid (DNS) color reagent with a spectrophotometer at 540 nm ($\epsilon = 1.31 \times 10^4$). The enzyme was added to one sample after the 30-min incubation for the blank control. Insoluble acid invertase activity in the fruit was calculated as the quantity of reducing sugars produced per min at 37°C. Each result shown was the mean of three replicated treatments.

Extraction and assay of SuSy and SPS: SuSy and SPS were extracted according to the methods of Hubbard *et al.* (1989) and Lowell *et al.* (1989), with some modifications. Frozen flesh was homogenized in a chilled mortar using a 1:5 tissue-to-buffer ratio. The buffer contained 100 mM phosphate buffer (pH 7.5), 5 mM MgCl₂, 1 mM EDTA, 2.5 mM DTT, 0.1% (v/v) Triton X-100, and 2% PVPP (w/v). The homogenates were centrifuged at $10,000 \times g$ for 30 min at 4°C. After centrifugation, the supernatants were dialyzed in a 15-cm dialysis tube (molecular weight cut-off of 8,000–15,000) for approximately 16 h at 4°C against a solution containing 10 mM phosphate buffer (pH 7.5), 0.5 mM MgCl₂, 0.1 mM EDTA, 0.25 mM DTT, and 0.01% (v/v) Triton X-100. The solution remaining in the dialysis tube was collected for analysis of crude SuSy and SPS activity. SPS activity was assayed by adding 0.1 mL of crude extract to 50 μ L Hepes buffer [50 mM Hepes–NaOH (pH 7.5), 15 mM MgCl₂, 15 mM fructose-6-PN₂, 15 mM glucose-6-PN₂, and 15 mM UDP-glucose]. The samples were incubated for 30 min at 37°C, after which the reaction was stopped by the addition of 0.2 mL of 30% (v/v) KOH. The tubes were placed in boiling water for 10 min to destroy any nonreacted fructose or fructose-6-P. After cooling, 3 mL of a mixture of 0.14% (w/v) anthrone in 13.8 M H₂SO₄ was added to each sample, and the samples were incubated in a 40°C-water bath for 20 min. After adding four volumes of 0.14% (w/v) anthrone reagent (in 14.6 M H₂SO₄), absorbance was

measured at 620 nm ($\epsilon = 1.96 \times 10^4$). The procedure for the SuSy assay (measured in the sucrose synthesis direction) was identical to that of SPS except that the reaction mixtures contained 0.1 M phosphate buffer (pH 8.0) and 60 mM fructose and did not contain fructose-6-P or glucose-6-P. Each result shown was the mean of three replicated treatments.

Statistical analysis: All the data in the present study were expressed as means \pm SE. Significance analysis was performed using *SAS* software (*SAS Institute, Inc.*, Cary, NC, USA). One-way analysis of variance (*ANOVA*) method (*Tukey's* multiple range test) was used to detect the significance ($P < 0.05$).

Results

P_N : The P_N of the grafted-root line was higher than that of the corresponding own-root line during the fruit development stage, and the difference between the grafted-root line and corresponding own-root line increased with the prolongation of fruit development time in both diploid and triploid watermelon (Fig. 2). These results indicate that pumpkin rootstock could improve photosynthesis of diploid and triploid watermelon.

Chl fluorescence parameters: During the fruit development stage, the F_v/F_m , Φ_{PSII} , and ETR of the grafted-root line were higher than those of the corresponding own-root line regardless of diploid watermelon or triploid watermelon (Fig. 3). The F_v/F_m , Φ_{PSII} , and ETR showed significant differences between the grafted-root line and own-root line during the fruit development stage in diploid watermelon (Fig. 3A,D,E). Meanwhile, the F_v/F_m , Φ_{PSII} , and ETR showed significant differences between the grafted-root line and own-root line during the late stage (30 DAP) of fruit development in triploid watermelon (Fig. 3B,C,F).

The activity of alkaline α -galactosidase in fruits

decreased with the prolongation of fruit development time for all watermelon lines. However, the alkaline α -galactosidase activity of the own-root line decreased sharper than that of the corresponding grafted-root line in both diploid watermelon and triploid watermelon. For diploid watermelon, the alkaline α -galactosidase activity in fruits of the grafted-root line was significantly higher than that in fruits of the own-root line during the fruit development stage (Fig. 4A). For triploid watermelon, the activity of alkaline α -galactosidase was higher in the grafted-root line than that in the own-root line during the fruit development stage, and the difference of alkaline α -galactosidase activity between the grafted-root line and own-root line was significant at the late development stage (30 DAP) (Fig. 4B).

Dry matter and mass accumulation of fruit: The changes in dry matter accumulation were similar to the changes in mass accumulation during fruit development for all watermelon lines (Fig. 5). Single fruit mass and dry matter content increased with the prolongation of fruit development time for all watermelon lines (Fig. 5). For diploid watermelon, the single fruit mass and dry matter content of the grafted-root line were significantly higher than those of the own-root line during fruit development (Fig. 5A,D). For triploid watermelon, the single fruit mass and dry matter content of the grafted-root line were higher than those of the own-root line at various stages of fruit development and the difference was significant during the late stage (30 DAP) of fruit development (Fig. 5B,C).

The activities of IAI, SPS, and SuSy: For diploid own-root line and corresponding grafted-root line, the IAI activity in flesh first increased sharply and then declined slightly; SuSy activity in flesh first declined slightly and then increased sharply, while SPS activity in flesh was increasing all the time during the fruit development stage (Fig. 6A,D,E). Meanwhile, the activities of IAI, SuSy, and SPS in flesh increased with the prolongation of fruit development time in triploid own-root line and

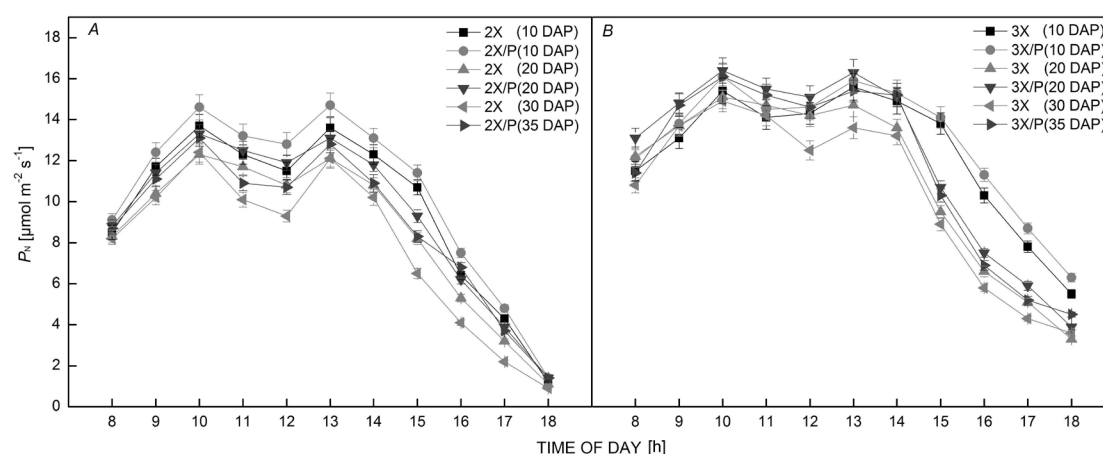


Fig. 2. Changes of net photosynthetic rate (P_N) in grafted-root watermelon lines and own-root watermelon lines during the fruit development stage. Error bars show SE, $n = 10$. DAP – days after pollination, 2X – diploid watermelon line, 3X – triploid watermelon line, 2X/P – diploid watermelon line grafted onto pumpkin rootstock, 3X/P – triploid watermelon line grafted onto pumpkin rootstock.

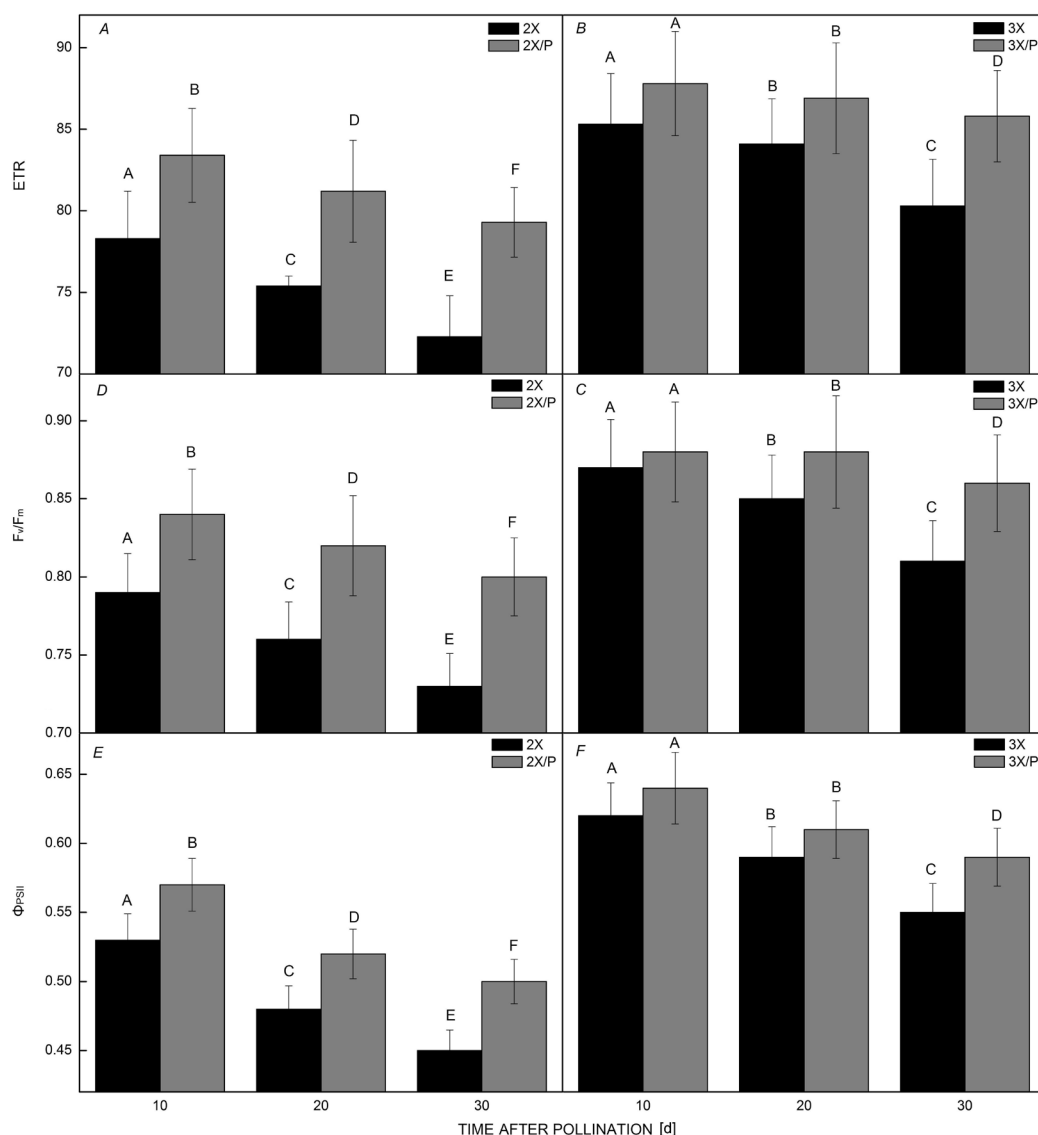


Fig. 3. Changes of chlorophyll fluorescence parameters in grafted-root watermelon lines and own-root watermelon lines during the fruit development stage. Error bars show SE, $n = 10$. One-way analysis of variance (ANOVA) was used to identify the difference between the grafted-root watermelon line and the corresponding own-root watermelon line in each treatment. Letters are comparable within grafted-root watermelon line and corresponding own-root watermelon line in each time. Values with *different* letters are significantly different ($P < 0.05$). DAP – days after pollination, 2X – diploid watermelon line, 3X – triploid watermelon line, 2X/P – diploid watermelon line grafted onto pumpkin rootstock, 3X/P – triploid watermelon line grafted onto pumpkin rootstock, F_v/F_m – maximum photochemical efficiency of PSII, Φ_{PSII} – actual photochemical efficiency of photosystem, ETR – electron transport rate.

corresponding grafted-root line (Fig. 6B,C,F). Both diploid watermelon and triploid watermelon, the activities of IAI, SPS, and SuSy were significantly lower in the grafted-root line than those in the corresponding own-root line during the fruit development stage (Fig. 6).

Sucrose and total sugar accumulation: The changes in total sugar accumulation were similar to the changes in sucrose accumulation during fruit development for all watermelon lines (Fig. 7). The contents of sucrose and total sugar in flesh increased with the prolongation of fruit development time for all watermelon lines (Fig. 7). The

sucrose and total sugar contents in the flesh of the grafted-root line were significantly lower than those in the flesh of own-root line regardless of diploid or triploid watermelon (Fig. 7).

Discussion

Mass and sucrose accumulation of crop fruit depends on the capacity of source tissues (especially leaves) to produce photoassimilates, during the fruit development process, as well as on the ability of sink tissues to unload these photoassimilates (Reynolds *et al.* 2012, Tuncel and Okita

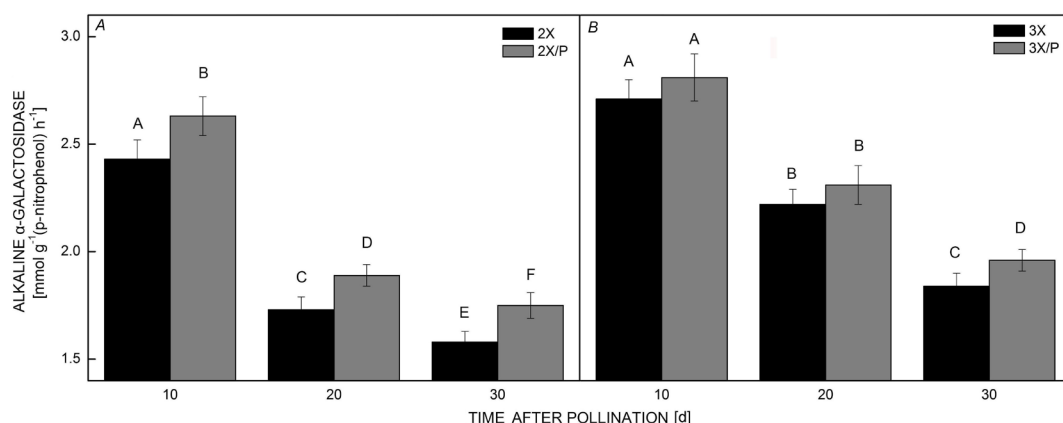


Fig. 4. Alkaline α -galactosidase activity in fruits of grafted-root watermelon lines and own-root watermelon lines during the fruit development stage. Error bars show SE, $n = 3$. One-way analysis of variance (ANOVA) was used to identify the difference between the grafted-root watermelon line and the corresponding own-root watermelon line in each treatment. Letters are comparable within grafted-root watermelon line and corresponding own-root watermelon line in each time. Values with *different letters* are significantly different ($P < 0.05$). DAP – days after pollination, 2X – diploid watermelon line, 3X – triploid watermelon line, 2X/P – diploid watermelon line grafted onto pumpkin rootstock, 3X/P – triploid watermelon line grafted onto pumpkin rootstock.

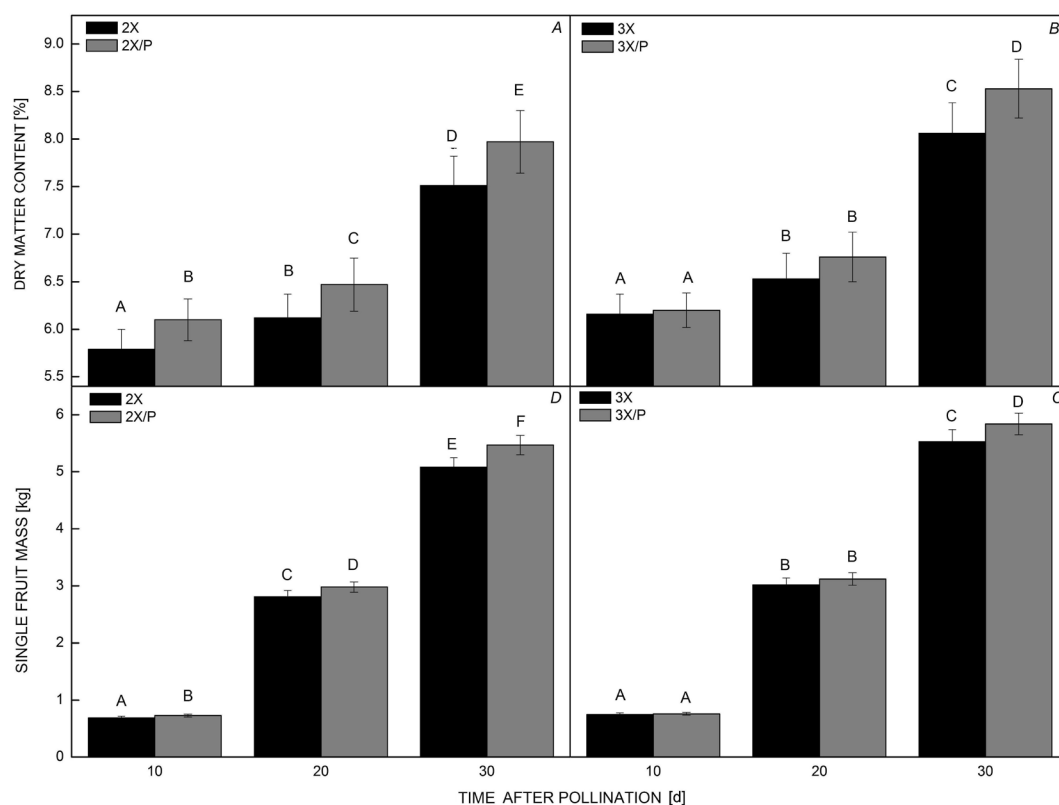


Fig. 5. Fruit mass and dry matter accumulation in grafted-root watermelon lines and own-root watermelon lines during the fruit development stage. Error bars show SE, $n = 10$. One-way analysis of variance (ANOVA) was used to identify the difference between the grafted-root watermelon line and the corresponding own-root watermelon line in each treatment. Letters are comparable within grafted-root watermelon line and corresponding own-root watermelon line in each time. Values with *different letters* are significantly different ($P < 0.05$). DAP – days after pollination, 2X – diploid watermelon line, 3X – triploid watermelon line, 2X/P – diploid watermelon line grafted onto pumpkin rootstock, 3X/P – triploid watermelon line grafted onto pumpkin rootstock.

2013). Photosynthesis is the physicochemical process to transduce light energy into chemical energy, and it provides the organic blocks for plant growth and development.

Theoretically, the yield and sugar content of crop fruit can be increased by promoting photosynthesis because photosynthesis is the major metabolic pathway that converts

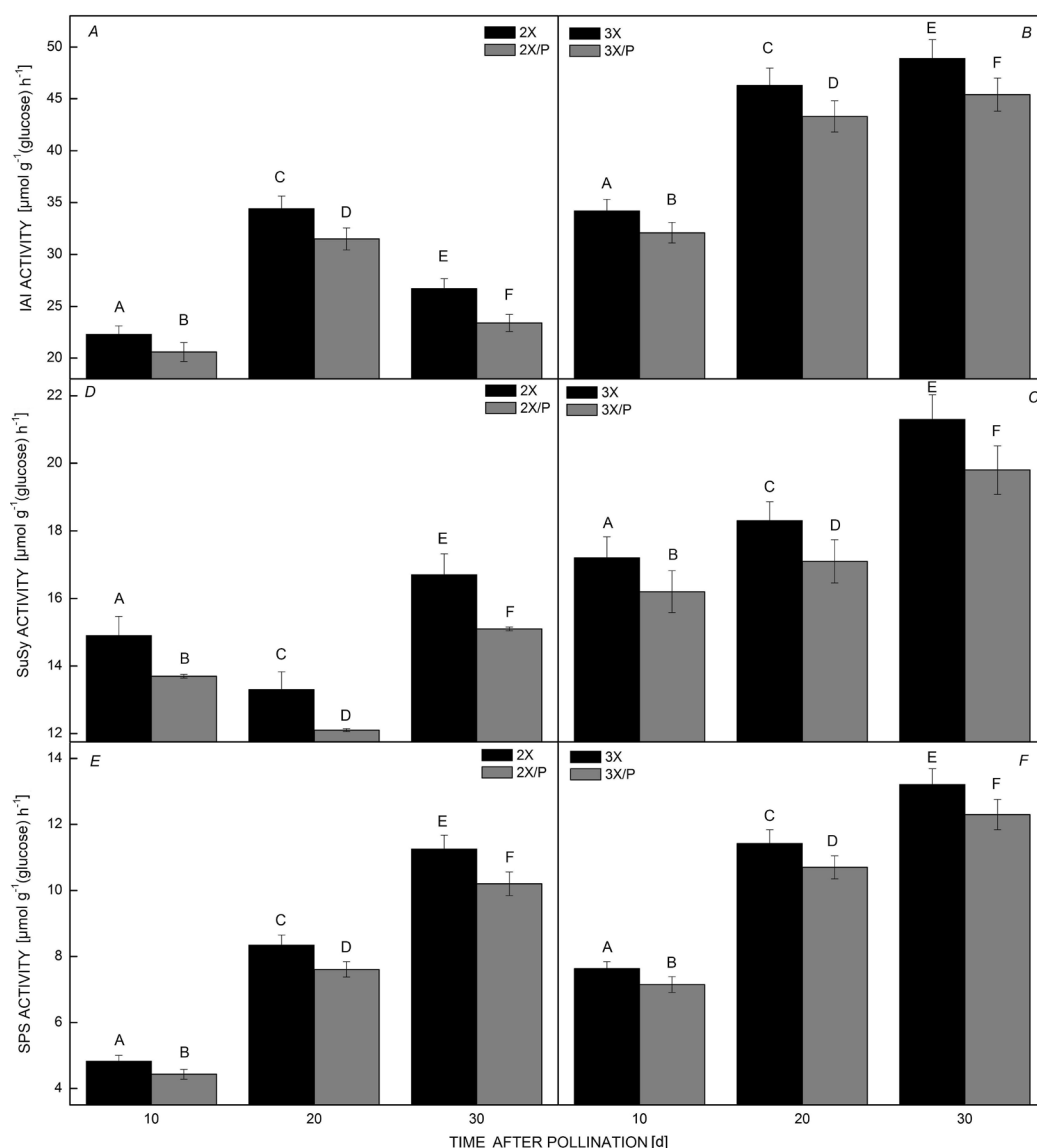


Fig. 6. The activities of insoluble acid invertase (IAI), sucrose phosphate synthase (SPS), and sucrose synthase (SuSy) in the flesh of grafted-root watermelon lines and own-root watermelon lines during the fruit development stage. Error bars show SE, $n = 3$. One-way analysis of variance (ANOVA) was used to identify the difference between the grafted-root watermelon line and the corresponding own-root watermelon line in each treatment. Letters are comparable within grafted-root watermelon line and corresponding own-root watermelon line in each time. Values with different letters are significantly different ($P < 0.05$). DAP – days after pollination, 2X – diploid watermelon line, 3X – triploid watermelon line, 2X/P – diploid watermelon line grafted onto pumpkin rootstock, 3X/P – triploid watermelon line grafted onto pumpkin rootstock.

carbon dioxide into organic compounds, such as fructose, glucose, sucrose, and starch in plants (Chang *et al.* 2017). Chl fluorescence provides a measure of the functional status of photosynthesis and has been used as sensitive indicator of plant photosynthetic performance (Naumann *et al.* 2007, Oukarroum *et al.* 2009). The increase in F_v/F_m , ETR, and Φ_{PSII} indicates a progressive upregulation in the quantum yield of photosynthesis, rates of carbon fixation, and photosynthetic efficiency (Oxborough 2004, Kalaji *et al.* 2011, Shu *et al.* 2013). The P_N , ETR, F_v/F_m , and Φ_{PSII} were higher in the rootstock-grafted line than that in corresponding own-root line during the fruit development

stage in both diploid watermelon and triploid watermelon (Figs. 2,3). These results suggest that pumpkin rootstock could improve photosynthetic capacity and efficiency of light energy utilization, which could improve the capacity of leaves to produce photoassimilates in rootstock-grafted watermelon lines. Various pumpkin rootstocks are more vigorous than cultivated watermelon varieties and responsible for the uptake of mineral nutrients and water in grafted plants (Petropoulos *et al.* 2012). Pumpkin-grafted watermelon plants have higher root volume, root surface area, and number of root tips and forks than corresponding own-root watermelon plants (Huang *et al.*

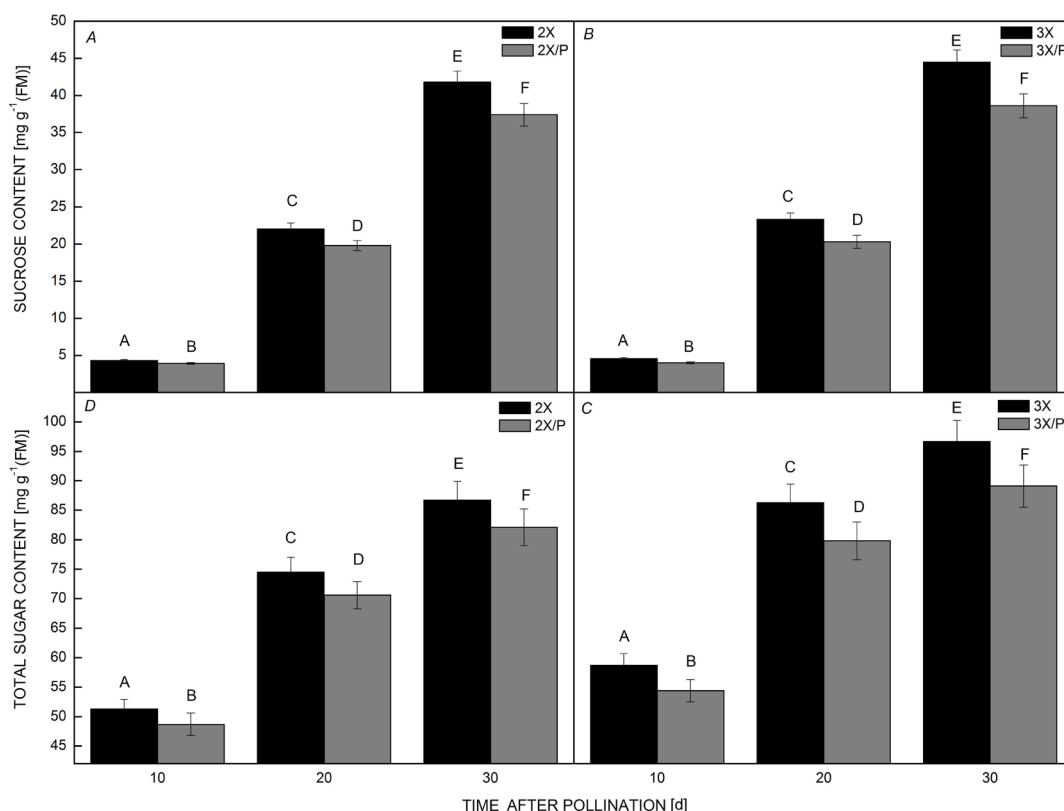


Fig. 7. Sucrose and total sugar accumulation in the flesh of grafted-root watermelon lines and own-root watermelon lines during the fruit development stage. Error bars show SE, $n = 5$. One-way analysis of variance (ANOVA) was used to identify the difference between the grafted-root watermelon line and the corresponding own-root watermelon line in each treatment. Letters are comparable within grafted-root watermelon line and corresponding own-root watermelon line in each time. Values with *different letters* are significantly different ($P < 0.05$). 2X – diploid watermelon line, 3X – triploid watermelon line, 2X/P – diploid watermelon line grafted onto pumpkin rootstock, 3X/P – triploid watermelon line grafted onto pumpkin rootstock.

2016a). A vigorous pumpkin rootstock can increase dry mass and mineral concentration (N, K, Ca, Mg, *etc.*) in watermelon leaves (Huang *et al.* 2016a). Mineral nutrition is essential for plant growth and is involved in virtually all metabolic and cellular functions. For example, Mg is an essential macronutrient that is required for important functions related to ATPase activity, ATP biosynthesis, chlorophyll synthesis, CO_2 assimilation, and phloem loading in leaves (Yang *et al.* 2012, Huang *et al.* 2016b). Leaves are the main photosynthetic organs, and the vigor of leaves and higher macronutrients concentration (N, K, Mg, *etc.*) in leaves is beneficial for photosynthesis of pumpkin-grafted watermelon. Raffinose oligosaccharides are the main photoassimilates that are translocated in the phloem of Cucurbitaceae family members (Zhang *et al.* 2010). Alkaline α -galactosidase is an important enzyme for raffinose oligosaccharides unloading and partitioning in cucumber and melon sink tissue (Pharr and Sox 1984, Gaudreault and Webb 1986, Gao and Schaffer 1999, Dai *et al.* 2011). Pumpkin rootstock improved alkaline α -galactosidase activity in both the diploid and the triploid watermelon line (Fig. 4). More photoassimilates could be allocated to grafted-root watermelon fruit than to corresponding own-root watermelon fruit. Pumpkin

rootstock could improve the synthesis of photoassimilates and their unloading into the fruit in both diploid and triploid watermelon. This may be the reason why pumpkin rootstock can increase fruit mass and dry matter content in both diploid and triploid watermelon.

Sucrose, glucose, and fructose are the main sugars in watermelon. Sucrose content increases rapidly over subsequent fruit developmental stage, while fructose and glucose contents remain almost constant during watermelon fruit development (Elmstrom and Davis 1981, Brown and Summers 1985, Kano 1991). Therefore, the difference in sugar content between watermelon cultivars is mainly determined by the sucrose content (Yativ *et al.* 2010). The sugar accumulation of crops depends on the ability of sink tissues to convert photoassimilates into sugar (Miralles and Slafer 2007, Reynolds *et al.* 2012, Tuncel and Okita 2013). The conversion capacity of photoassimilates to sucrose has become an important factor for sugar accumulation of watermelon fruit. The most well-studied enzymes that function in sucrose metabolism during fruit development include three enzyme families, *i.e.*, IAI, SuSy, and SPS. IAI is an extracellular enzyme that is bound to the cell wall (Karupiah *et al.* 1989, Iwatsubo *et al.* 1992). Both phloem unloading and

sucrose translocation to the developing sinks require IAI in sucrose-translocating plants (Godt and Roitsch 1997, Tang *et al.* 1999). SuSy is a key enzyme that catalyzes the synthesis of sucrose. A positive correlation between SuSy activity and sucrose accumulation was also reported in melon fruit (Burger and Schaffer 2007) and in watermelon (Yativ *et al.* 2010), suggesting that this enzyme plays an important role in determining sugar accumulation in sweet cucurbit fruit. SPS is another key enzyme that catalyzes sucrose synthesis. SPS is localized in the cytosol of the cells of many tissues including sink organs such as seeds and fruits (Huber and Huber 1996). SPS activity is positively related to sucrose accumulation in melon (Hubbard *et al.* 1989, Burger and Schaffer 2007) and watermelon (Yativ *et al.* 2010). In this experiment, although pumpkin rootstock increased P_N , ETR, F_v/F_m , Φ_{PSII} , and alkaline α -galactosidase activity, it decreased activities of IAI, SPS, and SuSy (Fig. 6). Previous studies have reported that physiological differences are strongly correlated with gene expression changes in the scion after grafting and some genes of the scion are upregulated or downregulated by rootstocks (Sun *et al.* 2020). In this study, the activities of IAI, SPS, and SuSy decreased in grafted watermelon lines vs. own-root watermelon lines. It remains possible that the genes encoding IAI, SPS, and SuSy in grafted watermelon lines were downregulated by pumpkin rootstock. The grafted plant always maintains the exchange of substances between scion and rootstock, for example, various types of plant RNA species move from cell to cell or systemically to potentially regulate grafted plant physiological processes (Lewsey *et al.* 2016, Huang *et al.* 2019). Pumpkins generally accumulate lower amounts of sugar in fruit than cultivated watermelon varieties. It could imply that some substances unfavorable to sugar synthesis from pumpkin rootstock move into watermelon scion, and inhibit expression of genes encoding IAI, SPS, and SuSy in the grafted watermelon lines. The lower activities of IAI, SPS, and SuSy would reduce the conversion of photoassimilates to sucrose in the grafted-root watermelon line. Sucrose accumulation was downregulated in the grafted-root line vs. corresponding own-root line in both diploid and triploid watermelon. As a result, sucrose and total sugar contents of the grafted-root line were lower than those of the corresponding own-root line in both diploid and triploid watermelon.

In conclusion, pumpkin rootstock increased P_N , ETR, F_v/F_m , and Φ_{PSII} during the fruit development stage in both the diploid watermelon line and the triploid watermelon line. These advantages could improve photosynthetic capacity, the utilization efficiency of light energy, and photosynthate assimilation in both the diploid and triploid watermelon lines. The activity of alkaline α -galactosidase was much higher in the grafted-root watermelon line than that in the corresponding own-root watermelon line during fruit development, which accelerated photoassimilate unloading and partitioning in grafted-root watermelon fruit. It was the reason why the dry matter content and mass of grafted-root lines were higher than those of the corresponding own-root line in both diploid and triploid watermelon fruit. On the other hand, pumpkin rootstock

decreased the activities of IAI, SPS, and SuSy during fruit development in both diploid and triploid watermelon flesh. The lower IAI, SPS, and SuSy activities were disadvantageous for converting photoassimilates into sucrose in the grafted-root watermelon lines, which caused that the sucrose and total sugar content in the flesh of the grafted-root line was lower than those in the flesh of corresponding own-root line regardless of diploid watermelon or triploid watermelon.

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