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The effects of arbuscular mycorrhizal fungi and deficit irrigation on the yield and sugar content of watermelons (*Citrullus lanatus*)

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Abstract: Many studies have demonstrated that arbuscular mycorrhizal fungi (AMF) and deficit irrigation (DI) have positive effects on the fruit yield or quality. This study aims to test whether the watermelon sugar content and yield can be improved by appropriate amounts of AMF and DI methods and to clarify the underlying physiological mechanism. Diploid and triploid watermelon cultivars and their pumpkin rootstock-grafted seedlings were treated with AMF, DI and DI + AMF in a randomised complete block design with five replications. The results showed that DI significantly reduced the relative water content (RWC), P_N, alkaline α-galactosidase activity, but increased the insoluble acid invertase (IAI), sucrose synthase (SuSy) and sucrose phosphate synthase (SPS) activities compared with the well-watered (WW) treatment, which led to a decrease in the fruit yield and an increase in the fruit sugar content. Although the AMF improved the RWC, P_N and alkaline α-galactosidase, IAI, SuSy and SPS activities in all the watermelon lines under both the DI and WW conditions, the improvement magnitude of these parameters was more pronounced in the pumpkin-root watermelon lines than the corresponding own-root watermelon lines, especially under the DI condition. The integrated application of AMF and DI increased the fruit yield to a level similar to the WW value in the pumpkin-root watermelon lines and sugar content to an optimal level in all the watermelon lines.

Keywords: pumpkin-root; relative water content; alkaline α-galactosidase; insoluble acid invertase; sucrose synthase; sucrose phosphate synthase

Watermelons (*Citrullus lanatus*) are an important crop and watermelon cultivars are either diploid or triploid. However, both diploid cultivars and triploid cultivars are susceptible to soil-borne pathogens (Lin et al. 2013). To date, pumpkin rootstock grafting has widely been used in watermelons to limit the effects of soil-borne pathogens (Shireen et al. 2020).

The supply of water in the root zone can affect both the crop yield and quality as water is a major determinant of the yield and quality (Carrijo et al. 2017). Deficit irrigation (DI) involve the supply of water below the full or optimum amount required by the crop for its physiological functions and might lead to yield penalties and an upturn in quality (Adu et al. 2018; Jovanovic, Stikic 2018). For some crops, a premium is placed on certain quality attributes and, therefore, trade-offs or yield reductions under reduced irrigation could be accommodated as long as it is compensated for by some improvement in desirable quality parameters. Hence, the application of DI serves the purpose of improving the quality of the

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fruit (Jovanovic, Stikic 2018; Faghih et al. 2019; Ezzo et al. 2020; Ghannem et al. 2021).

Arbuscular mycorrhizal fungi (AMF), a group of soil fungi, are able to colonise and establish symbiotic mutually beneficial associations with the roots of most agricultural crops (Gianinazzi, Vosátka 2004). In crop production, AMF have been used to increase the tolerance to biotic and abiotic stresses, enhance the nutrient uptake, and improve the water-use efficiency (Motaleb et al. 2020; Kazadi et al. 2022).

The yield and quality are the main traits of crops, and sweetness is a key attribute that determines the watermelon fruit's quality. There is a limited availability of data in studies on the underlying mechanisms for the variations in the sugar content and yield of watermelons under DI and AMF inoculation. This research aims to elucidate how DI and AMF induce variations in the yield and sugar content of different ploidy watermelons and their pumpkin rootstock-grafted seedlings and to clarify the underlying physiological mechanisms.

MATERIAL AND METHODS

Material. In this experiment, the commercial pumpkin rootstock cultivar 'Baimi112' (*Cucurbita maxima*), the commercial triploid watermelon cultivar 'Zheng No.3 (3X)' and the corresponding diploid watermelon line 'Zheng No.3 (2X)' [*Citrullus lanatus* (Thunb.) Matsum. and Nakai.] were used. The AMF (*Rhizophagus intraradices*) was provided by Henan Agricultural University, Zhengzhou, People's Republic of China. Wich was multiplied in a pot culture for 15 weeks in greenhouse conditions using maize. The average colonisation of *Rhizophagus intraradices* was 95.6% and the average spore density was 549 per 10 g of air-dried soil. The spores, mycelium, colonised root fragments, and dried sand-soil were mixed to use as AMF inoculums.

Experimental design. The pumpkin and watermelon seeds were sterilised with 3% (v/v) sodium hypochlorite for 10 min, rinsed with distilled water and soaked for 5 h, then placed in a dark growth chamber at 30 °C for germination. The uniformly germinated 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 with a 14/10 h day/night photoperiod at temperatures ranging between approximately 22 and 30 °C at an ambient relative humidity. Two grafting combinations of watermelon lines were used, that is, the triploid watermelon (3X) was grafted onto the pumpkin (3X/P) and the diploid watermelon (2X) was grafted onto the pumpkin (2X/P). Once the pumpkin rootstock seedling produced the first true leaf, grafting was performed by using the hole-insertion grafting method as described by Hassell et al. (2008). When the third true leaf emerged, the own-root and grafted-root watermelon seedlings were transplanted into a rain sheltered greenhouse under natural daylight conditions in Xinxiang, China (35°18'N, 113°52'E) in early May. The daily mean highest/lowest temperatures in May, June and July were 29/18 °C, 34/23 °C and 34/26 °C, while the mean relative humidity was 56.7%, 51.9% and 69.3%, respectively. The soil characteristics were pH = 8.15 (soil : water ratio of 1 : 5, w/v); organic matter (15.6 g/kg); available nitrogen (36.8 mg/kg); available phosphorus (6.72 mg/kg); potassium (88.7 mg/kg). Each line comprised two rows, and each row was for ten individuals. The spacing between the rows was 200 cm, and the spacing between the individuals in a row was 100 cm. All the treatments were arranged in a randomised complete block design with five replications. The treatments were: (1) well-watered and non-AMF (WW); (2) well-watered and inoculated with AMF (WW + AMF); (3) deficit irrigation and non-AMF (DI); (4) deficit irrigation and inoculated with AFM (DI + AMF). The flowers were hand-pollinated and tagged. Five individual fruits were chosen randomly from each line of each treatment at 10, 20, 30 days after pollination and used to test the fruit mass, 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 the 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 the individual fruit.

Mycorrhizal inoculation and irrigation regime. The mycorrhizal inoculums were placed 15 cm below the seedlings at the time of transplanting (15 g per plant). The plants were carefully watered as needed to maintain the soil moisture near field capacity (80%). The DI treatments started five days after pollination and were imposed by withholding water from the plots until the soil water potential was achieved. At the same time, the well-watered plots were controlled with 80% of the field capacity (−0.075 MPa), and the DI plots were controlled

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with 60% of the field capacity (–0.14 MPa). The soil water potential was measured by a pressure plate apparatus (Shimadzu, CL–800, Kyoto, Japan) and the amount of water loss was supplied to each plot to keep the intended soil water contents.

Relative water content. The relative water content (RWC) was determined on fresh leaf (the third leaf from the top) discs, 2 cm in diameter, which was calculated according to Hayat et al. (2007). Each result is the mean of 5 replicate treatments.

Net photosynthetic rate (P_N). An LI6400 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 days after pollination, which were measured at 10:00 a.m. each of the specified days. Each result is the mean of 5 replicate treatments.

Sucrose and total sugar determination. The sucrose and total sugar content was assayed as described by Liu et al. (2013).

Assay of enzymatic activity. The alkaline α-galactosidase activity was assayed according to the method of Gao and Schaffer (1999). The insoluble acid invertase activity was measured according to the method of Miron and Schaffer (1991). The sucrose synthase and sucrose phosphate synthase were extracted and assayed according to the methods of Liu et al. (2013). Each result is the mean of five replicate treatments.

Statistical analysis. A significance analysis was performed using SAS software (SAS Institute, Inc., Cary, NC, USA). A two-way analysis of variance (ANOVA) method (Tukey's multiple range test) was used to detect the significance ($P < 0.05$).

RESULTS

At the fruit development stage, the arbuscular mycorrhizal fungi (AMF) slightly improved, P_N, alkaline α-galactosidase activity and fruit yield in the well-watered (WW) watermelon lines (Tables 1 and 2). The deficit irrigation significantly ($P < 0.05$) decreased these parameters compared with the WW treatment (Tables 1 and 2). The arbuscular mycorrhizal fungi significantly ($P < 0.05$) improved these parameters in the DI watermelon lines, but remained lower than the values of the corresponding WW plants, and the improvement magnitudes of these parameters were greater in the pumpkin-root line compared with the corresponding own-root lines. The the relative water content (RWC), P_N, alkaline α-galactosidase activity and fruit yield of the pumpkin-root lines showed no significant differences between the WW treatment and the DI + AFM treatment. However, the difference of these parameters was significant in the own-root lines (Tables 1 and 2).

The arbuscular mycorrhizal fungi improved the activity of the insoluble acid invertase (IAI), sucrose phosphate synthase (SPS) and sucrose synthase (SuSy), the sucrose content and the total sugar content in the WW plants, but did not significantly affect these parameters (Tables 3 and 4). The deficit irrigation significantly ($P < 0.05$) increased these parameters in all the watermelon lines compared with the WW treatment, and the AMF further improved these parameters in the DI lines (Tables 3 and 4). The arbuscular mycorrhizal fungi increased these parameters more dramatically in the pumpkin-root watermelon line than in the corresponding own-root watermelon line under both the WW and DI treatments, especially under the DI condition (Tables 3 and 4). The activity of IAI, SPS and SuSy, the sucrose content and the total sugar content in the pumpkin-root line were lower than those in the corresponding own-root line under the WW, WW + AMF and DI treatments. However, these parameters in the pumpkin-root line were higher under the DI + AMF treatment (Tables 3 and 4). The sucrose content and total sugar content were highest in the pumpkin-root triploid line among all the watermelon lines under the DI + AMF treatment.

DISCUSSION

Water is one of the most important factors affecting the yield and quality of crop products. For example, the highest yield was obtained from more frequent irrigation, but water deficit might lead to yield penalties and an upturn in the proportion of sugars in the fruits (Sensoy et al. 2007; Adu et al. 2018). The yield and sugar accumulation of fruit crop depend on the capacity of the source tissues to produce photoassimilates, as well as on the ability of the sink tissues to unload these photoassimilates, and the conversion capacity of the photoassimilates to sugar. Photosynthesis is the major metabolic pathway that converts carbon dioxide (CO_2) into organic compounds in plants (Chang et al. 2017). Raffinose oligosaccharides are the main photoassimilates that are translocated in the phloem of *Cucurbitaceae* family members (Zhang et al. 2010). Alkaline

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Table 1. The RWC (%), P_N (mmol/m^2.s) and alkaline α-galactosidase activity [mmol/g (p-nitrophenol)·h] under different treatments during the fruit development stage (mean ± SE)

Traits	Stages	Treatments	2X	2X/P	3X	3X/P
RWC	10 DAP	WW	92.68 ± 3.7^{ab}	93.59 ± 4.1^{ab}	93.81 ± 3.5^{ab}	95.49 ± 3.5^{b}
		WW + AMF	93.22 ± 2.2^{ab}	94.67 ± 3.7^{ab}	94.72 ± 2.1^{ab}	96.84 ± 2.3^{b}
		DI	75.38 ± 2.8^{c}	84.72 ± 2.9^{de}	81.29 ± 2.8^{df}	87.21 ± 3.2^{eg}
		DI + AMF	79.59 ± 3.5^{f}	90.87 ± 3.4^{ag}	86.41 ± 3.6^{e}	94.28 ± 2.9^{ab}
	20 DAP	WW	90.32 ± 3.8^{ab}	91.78 ± 2.2^{abc}	92.21 ± 2.7^{abc}	93.49 ± 3.7^{bc}
		WW + AMF	91.61 ± 4.2^{abc}	93.86 ± 3.5^{bc}	94.12 ± 2.2^{bc}	95.78 ± 3.5^{c}
		DI	76.69 ± 3.3^{d}	81.29 ± 2.7^{e}	80.89 ± 3.5^{e}	83.65 ± 2.8^{ef}
		DI + AMF	80.92 ± 1.8^{e}	88.31 ± 2.8^{ag}	85.91 ± 2.8^{fg}	91.62 ± 4.1^{abc}
	30 DAP	WW	83.38 ± 2.8^{ab}	90.61 ± 3.6^{cde}	91.18 ± 3.6^{cde}	92.11 ± 2.7^{de}
		WW + AMF	84.05 ± 3.4^{ab}	92.13 ± 2.1^{de}	92.31 ± 2.4^{de}	93.85 ± 4.2^{e}
		DI	69.29 ± 2.7^{f}	80.59 ± 3.2^{ag}	77.78 ± 3.3^{g}	82.26 ± 3.6^{a}
		DI + AMF	73.21 ± 3.2^{h}	86.87 ± 2.8^{bc}	82.91 ± 3.1^{ab}	89.09 ± 2.1^{cd}
P_N	10 DAP	WW	13.69 ± 0.53^{a}	14.43 ± 0.68^{bc}	15.39 ± 0.72^{de}	16.31 ± 0.67^{fg}
		WW + AMF	13.91 ± 0.47^{ab}	14.87 ± 0.71^{cd}	15.82 ± 0.68^{ef}	16.89 ± 0.73^{g}
		DI	8.96 ± 0.36^{h}	11.53 ± 0.49^{i}	10.39 ± 0.49^{j}	13.28 ± 0.46^{a}
		DI + AMF	9.72 ± 0.41^{k}	13.82 ± 0.56^{ab}	11.78 ± 0.51^{i}	15.97 ± 0.63^{ef}
	20 DAP	WW	12.61 ± 0.52a	13.51 ± 0.53^{bc}	14.19 ± 0.57^{d}	15.71 ± 0.57^{ef}
		WW + AMF	12.82 ± 0.46^{a}	13.89 ± 0.61^{cd}	14.52 ± 0.63^{d}	16.28 ± 0.66^{f}
		DI	7.62 ± 0.27^{g}	9.32 ± 0.38^{h}	9.12 ± 0.35^{hi}	10.96 ± 0.51^{j}
		DI +AMF	8.69 ± 0.32^{i}	12.87 ± 0.57^{ab}	11.28 ± 0.47^{j}	15.32 ± 0.68^{e}
	30 DAP	WW	10.41 ± 0.48^{a}	12.01 ± 0.48^{bc}	13.89 ± 0.61^{d}	14.49 ± 0.58^{de}
		WW + AMF	10.59 ± 0.36^{a}	12.33 ± 0.52^{c}	14.21 ± 0.46^{d}	15.02 ± 0.37^{e}
		DI	5.78 ± 0.21^{f}	8.58 ± 0.27^{g}	8.13 ± 0.32^{h}	10.47 ± 0.42^{a}
		DI +AMF	6.38 ± 0.19^{i}	11.52 ± 0.41^{b}	9.59 ± 0.37^{j}	14.21 ± 0.61^{d}
Alkaline α-galactosidase	10 DAP	WW	2.43 ± 0.09^{a}	2.62 ± 0.11^{bc}	2.64 ± 0.09^{bc}	2.86 ± 0.12^{ed}
		WW + AMF	2.48 ± 0.10^{a}	2.71 ± 0.10^{cf}	2.71 ± 0.11^{cf}	2.97 ± 0.10^{d}
		DI	1.59 ± 0.06^{g}	1.91 ± 0.07^{h}	1.82 ± 0.07^{i}	2.11 ± 0.08^{j}
		DI + AMF	1.82 ± 0.08^{hi}	2.52 ± 0.09^{ab}	2.13 ± 0.09^{j}	2.79 ± 0.13^{ef}
	20 DAP	WW	1.73 ± 0.06^{a}	1.82 ± 0.06^{bc}	1.93 ± 0.06^{de}	2.15 ± 0.08^{fg}
		WW + AMF	1.75 ± 0.07^{ab}	1.87 ± 0.05^{cd}	1.97 ± 0.05^{e}	2.23 ± 0.09^{g}
		DI	1.07 ± 0.04^{h}	1.28 ± 0.04^{i}	1.26 ± 0.04^{ij}	1.54 ± 0.05^{k}
		DI + AMF	1.21 ± 0.05^{j}	1.74 ± 0.05^{ab}	1.48 ± 0.07^{k}	2.11 ± 0.07^{f}
	30 DAP	WW	1.61 ± 0.07^{a}	1.72 ± 0.06^{bc}	1.74 ± 0.06^{cd}	1.81 ± 0.04^{de}
		WW + AMF	1.64 ± 0.05^{ab}	1.77 ± 0.05^{cd}	1.78 ± 0.04^{cd}	1.87 ± 0.07^{e}
		DI	0.98 ± 0.03^{f}	1.19 ± 0.03^{g}	1.11 ± 0.03^{h}	1.27 ± 0.05^{i}
		DI + AMF	1.08 ± 0.05^{h}	1.64 ± 0.06^{ab}	1.32 ± 0.03^{i}	1.76 ± 0.06^{cd}

$^{a–i}$ different letters are significantly different ($P < 0.05$) in each stage

RWC – relative water content; DAP – days after pollination; WW – the well-watered; DI – the deficit irrigation; WW + AMF – well-watered and inoculated with arbuscular mycorrhizal fungi; DI + AMF – deficit irrigation and inoculated with arbuscular mycorrhizal fungi; 2X – the diploid watermelon; 2X/P – the diploid watermelon grafted onto the pumpkin; 3X – the triploid watermelon; 3X/P – the triploid watermelon grafted onto the pumpkin

α-galactosidase is an important enzyme for raffinose oligosaccharides unloading and partitioning of sink tissue in *Cucurbitaceae* family crops (Dai et al. 2011). Photosynthesis is known to be very sensitive to water deficits. As the RWC is reduced, many plants show reductions in the P_N (Dias, Brüggemann 2007). In this

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Table 2. Fruit mass (kg) accumulation under different treatments during the fruit development stage (mean ± SE)

Stages	Treatments	2X	2X/P	3X	3X/P
10 DAP	WW	0.659 ± 0.028^{a}	0.741 ± 0.031^{bc}	0.758 ± 0.032^{cd}	0.801 ± 0.030^{ef}
	WW + AMF	0.672 ± 0.031^{a}	0.762 ± 0.028^{cd}	0.776 ± 0.029^{cde}	0.829 ± 0.036^{f}
	DI	0.541 ± 0.024^{g}	0.652 ± 0.024^{h}	0.631 ± 0.027^{h}	0.708 ± 0.028^{ab}
	DI + AMF	0.574 ± 0.023^{i}	0.712 ± 0.032^{ab}	0.687 ± 0.023^{a}	0.786 ± 0.032^{de}
20 DAP	WW	2.808 ± 0.11^{a}	2.979 ± 0.11^{bcd}	3.021 ± 0.12^{cd}	3.181 ± 0.14^{ef}
	WW + AMF	2.859 ± 0.12^{ab}	3.061 ± 0.13^{de}	3.085 ± 0.13^{de}	3.287 ± 0.12^{f}
	DI	2.262 ± 0.07^{h}	2.612 ± 0.09^{i}	2.529 ± 0.08^{ij}	2.809 ± 0.09^{a}
	DI + AMF	2.411 ± 0.08^{j}	2.878 ± 0.12^{abc}	2.778 ± 0.11^{a}	3.122 ± 0.12^{de}
30 DAP	WW	5.178 ± 0.21^{a}	5.669 ± 0.22^{bc}	5.731 ± 0.21^{c}	6.168 ± 0.27^{de}
	WW + AMF	5.281 ± 0.22^{a}	5.841 ± 0.25^{cf}	5.859 ± 0.19^{cf}	6.371 ± 0.22^{d}
	DI	4.019 ± 0.13^{h}	4.779 ± 0.18^{i}	4.748 ± 0.20^{i}	5.261 ± 0.21^{a}
	DI + AMF	4.278 ± 0.16^{j}	5.412 ± 0.21^{ab}	5.188 ± 0.23^{a}	6.032 ± 0.26^{ef}

$^{a–j}$ different letters are significantly different ($P < 0.05$) in each stage

DAP – days after pollination; WW – the well-watered; DI – the deficit irrigation; WW + AMF – well-watered and inoculated with arbuscular mycorrhizal fungi; DI + AMF – deficit irrigation and inoculated with arbuscular mycorrhizal fungi; 2X – the diploid watermelon; 2X/P – the diploid watermelon grafted onto the pumpkin; 3X – the triploid watermelon; 3X/P – the triploid watermelon grafted onto the pumpkin

study, the DI reduced the RWC leading to a decrease in the P_N, and this reduction was accompanied with a decrease in the alkaline α-galactosidase activity in the fruits (Table 1). These disadvantages could reduce the synthase of the photoassimilates in the leaf and unloading of the photoassimilates in the fruits, leading to a decrease in the fruit yield (Table 2).

Many studies suggest that an appropriate DI level improves the sugar content in a number of fruit crops, but the biochemical mechanism involved in the sugar assimilation within the watermelon plants need further studies (Sensoy et al. 2007; Ezzo et al. 2020). The sugar content of the watermelon is mainly determined by the sucrose content (Yativ et al. 2010; Liu et al. 2013). The most well-studied enzymes that function in the sucrose metabolism during the fruit development include three enzyme families, i.e., IAI, SuSy and SPS (Iwatsubo et al. 1992). Insoluble acid invertase is an extracellular enzyme that the phloem unloading and sucrose translocation to the developing sinks require IAI in plants (Godt, Roitsch 1997). Sucrose synthase and sucrose phosphate synthase are key enzymes that catalyse the synthesis of sucrose in watermelon fruit, and their activities are positively related to the sucrose accumulation in the fruit of watermelons (Yativ et al. 2010). In this study, the activities of IAI, SPS and SuSy increased significantly with the application of the DI (Table 3). The higher activities in the IAI, SPS and SuSy are beneficial to improve the conversion of the photoassimilates to sucrose in the DI watermelon line. Therefore, sucrose accumulation was up-regulated in the DI line versus the WW line. As a result, the sucrose and total sugar contents of the DI lines were higher than those of the corresponding WW lines (Table 4).

Arbuscular mycorrhizal fungi are able to colonise and establish symbiotic mutually beneficial associations with the roots of most agricultural crops and increase the effective absorptive area of the roots that enhance the efficiency in the absorption of nutrients and water (Cartmilla et al. 2012; Mathur et al. 2019; Kazadi et al. 2022). In this study, the AMF improved the RWC, PN and the activities of the alkaline *α*-galactosidase, IAI, SuSy and SPS under both the WW and DI conditions, which contributed to improving the yield and sugar content of the watermelon fruit (Tables 1–4). However, the AMF did not significantly alter these parameters in the WW watermelon line, but significantly increased these parameters in the DI watermelon line (Tables 1–4). It may be that a sufficient amount of water relieves the difference between the mycorrhizal line and non-mycorrhizal line. Moreover, although the AMF increased these parameters in all the watermelon lines under both the WW and DI conditions, the magnitude of the improvements was greater in the pumpkin-root line than in the corresponding own-root

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Table 3. The IAI, SPS and SuSy activity [μmol/g (glucose) h] under different treatments during the fruit development stage (mean ± SE)

Traits	Stages	Treatments	2X	2X/P	3X	3X/P
IAI	10 DAP	WW	22.31 ± 0.93^{a}	20.19 ± 0.85^{b}	25.68 ± 0.86^{de}	22.79 ± 0.87^{ac}
		WW + AMF	22.69 ± 0.87^{ac}	20.78 ± 0.93^{b}	26.31 ± 1.06^{de}	23.68 ± 0.95^{c}
		DI	23.82 ± 0.63^{c}	23.31 ± 1.05^{ac}	28.09 ± 1.13^{f}	26.81 ± 1.13^{ef}
		DI + AMF	25.38 ± 1.07^{d}	26.52 ± 1.13^{de}	30.81 ± 1.25^{g}	31.12 ± 1.21^{g}
	20 DAP	WW	34.38 ± 1.24^{a}	31.61 ± 1.23^{b}	41.28 ± 1.75^{gh}	36.69 ± 1.54^{de}
		WW + AMF	34.92 ± 1.35^{ac}	32.75 ± 1.45^{b}	42.51 ± 1.36^{hi}	38.31 ± 1.32^{ef}
		DI	36.59 ± 1.52^{cde}	36.09 ± 1.76^{acd}	44.39 ± 1.97^{i}	43.52 ± 1.87^{i}
		DI + AMF	38.91 ± 0.95^{f}	40.18 ± 1.58^{fg}	48.58 ± 1.06^{j}	49.18 ± 2.13^{j}
	30 DAP	WW	27.81 ± 1.17^{ac}	25.69 ± 1.07^{b}	31.89 ± 1.23^{e}	28.78 ± 1.07^{cd}
		WW + AMF	28.26 ± 1.25^{c}	26.61 ± 0.97^{ab}	32.68 ± 1.45^{ef}	30.09 ± 0.98^{d}
		DI	30.19 ± 1.22^{d}	28.89 ± 1.14^{cd}	35.11 ± 1.52^{g}	33.92 ± 1.52^{fg}
		DI + AMF	32.28 ± 1.53^{ef}	33.12 ± 1.53^{ef}	38.72 ± 1.21^{h}	39.31 ± 1.88^{h}
SPS	10 DAP	WW	5.21 ± 0.18^{a}	4.76 ± 0.17^{b}	6.27 ± 0.25^{ef}	5.51 ± 0.21^{cd}
		WW + AMF	5.29 ± 0.21^{ac}	4.93 ± 0.22^{b}	6.42 ± 0.28^{ef}	5.75 ± 0.24^{d}
		DI	5.72 ± 0.23^{d}	5.68 ± 0.23^{d}	6.97 ± 0.31^{g}	6.85 ± 0.32^{g}
		DI+AMF	6.12 ± 0.26^{e}	6.51 ± 0.27^{f}	7.88 ± 0.34^{h}	7.96 ± 0.35^{h}
	20 DAP	WW	8.34 ± 0.27^{a}	7.61 ± 0.32^{b}	9.42 ± 0.42^{de}	8.64 ± 0.38^{ac}
		WW + AMF	8.48 ± 0.36^{a}	7.84 ± 0.28^{b}	9.64 ± 0.37^{ef}	8.96 ± 0.32^{c}
		DI	9.02 ± 0.35^{cd}	8.75 ± 0.41^{ac}	10.61 ± 0.48^{g}	10.12 ± 0.46^{fg}
		DI + AMF	9.69 ± 0.41^{ef}	9.93 ± 0.36^{f}	11.56 ± 0.52^{h}	11.81 ± 0.53^{h}
	30 DAP	WW	12.31 ± 0.42^{a}	11.39 ± 0.47^{b}	14.61 ± 0.62^{e}	13.89 ± 0.61^{d}
		WW + AMF	12.48 ± 0.55^{a}	11.71 ± 0.52^{b}	14.88 ± 0.58^{e}	14.38 ± 0.58^{de}
		DI	13.19 ± 0.41^{c}	12.78 ± 0.58^{ac}	16.09 ± 0.71^{f}	15.81 ± 0.67^{f}
		DI + AMF	14.28 ± 0.35^{de}	14.89 ± 0.35^{e}	18.12 ± 0.42^{g}	18.72 ± 0.45^{g}
SuSy	10 DAP	WW	15.71 ± 0.54^{ac}	14.79 ± 0.63^{b}	17.21 ± 0.75^{fgh}	16.28 ± 0.62^{cde}
		WW + AMF	16.02 ± 0.63^{cd}	15.21 ± 0.71^{ab}	17.59 ± 0.67^{ghi}	16.91 ± 0.71^{efg}
		DI	16.68 ± 0.58^{def}	16.32 ± 0.58^{cde}	18.62 ± 0.87^{j}	18.22 ± 0.82^{ij}
		DI + AMF	17.79 ± 0.42^{hij}	18.48 ± 0.75^{j}	20.22 ± 0.46^{k}	20.79 ± 0.48^{k}
	20 DAP	WW	13.31 ± 0.56^{ab}	12.68 ± 0.56^{b}	15.32 ± 0.67^{gh}	14.28 ± 0.57^{de}
		WW + AMF	13.48 ± 0.61^{ac}	13.11 ± 0.47^{ab}	15.68 ± 0.58^{hi}	14.86 ± 0.64^{efg}
		DI	14.21 ± 0.65^{de}	14.02 ± 0.62^{cd}	16.64 ± 0.72^{j}	16.12 ± 0.72^{ij}
		DI + AMF	15.19 ± 0.42^{fgh}	15.81 ± 0.73^{hi}	17.93 ± 0.69^{k}	18.41 ± 0.83^{k}
	30 DAP	WW	17.41 ± 0.58^{ac}	16.48 ± 0.68^{b}	19.42 ± 0.66^{fgh}	18.18 ± 0.76^{cde}
		WW + AMF	17.62 ± 0.82^{acd}	17.02 ± 0.73^{ab}	19.78 ± 0.75^{ghi}	18.91 ± 0.89^{efg}
		DI	18.49 ± 0.75^{def}	18.21 ± 0.84^{cde}	20.81 ± 0.93^{j}	20.32 ± 0.67^{hij}
		DI + AMF	19.68 ± 0.45^{ghi}	20.52 ± 0.47^{ij}	22.59 ± 0.52^{k}	23.21 ± 0.56^{k}

$^{a–k}$ different letters are significantly different ($P < 0.05$) in each stage

IAI – insoluble acid invertase; SPS – sucrose phosphate synthase; SuSy – sucrose synthase; DAP – days after pollination; WW – the well-watered; DI – the deficit irrigation; WW + AMF – well-watered and inoculated with arbuscular mycorrhizal fungi; DI + AMF – deficit irrigation and inoculated with arbuscular mycorrhizal fungi; 2X – the diploid watermelon; 2X/P – the diploid watermelon grafted onto the pumpkin; 3X – the triploid watermelon; 3X/P – the triploid watermelon grafted onto the pumpkin

line. These results suggest that the pumpkin root and watermelon root responded differently to the AMF. Various pumpkin rootstocks have a higher root volume, root surface area, and number of root tips than watermelon roots (Huang et al. 2016). We reasoned that the vigorous roots of the pumpkin rootstocks

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Table 4. Sucrose and total sugar content [mg/g (FW)] under different treatments during the fruit development stage (mean ± SE)

Traits	Stages	Treatments	2X	2X/P	3X	3X/P
Sucrose content	10 DAP	WW	4.809 ± 0.21^{a}	4.759 ± 0.17^{a}	5.331 ± 0.21^{efg}	5.059 ± 0.21^{bcd}
		WW + AMF	4.871 ± 0.19^{abc}	4.851 ± 0.16^{ab}	5.412 ± 0.19^{fg}	5.182 ± 0.19^{def}
		DI	5.102 ± 0.16^{cde}	5.132 ± 0.21^{de}	5.702 ± 0.22^{hi}	5.552 ± 0.22^{gh}
		DI + AMF	5.212 ± 0.22^{def}	5.416 ± 0.19^{fg}	5.878 ± 0.17^{i}	5.921 ± 0.17^{i}
	20 DAP	WW	22.59 ± 0.87^{ac}	21.31 ± 0.96^{b}	25.49 ± 1.05^{fgh}	24.18 ± 0.96^{de}
		WW + AMF	22.86 ± 0.69^{ac}	21.79 ± 0.68^{ab}	25.97 ± 0.93^{gh}	24.92 ± 0.87^{efg}
		DI	24.11 ± 0.93^{de}	23.34 ± 0.57^{cd}	27.51 ± 0.65^{ij}	26.67 ± 1.06^{hi}
		DI + AMF	24.39 ± 0.58^{def}	24.68 ± 1.06^{ef}	28.12 ± 1.12^{j}	28.47 ± 1.21^{j}
	30 DAP	WW	41.79 ± 1.72^{abc}	40.38 ± 1.35^{b}	44.51 ± 1.97^{efg}	42.81 ± 1.95^{acde}
		WW + AMF	42.31 ± 1.23^{abcd}	41.31 ± 1.62^{ab}	45.32 ± 1.76^{fgh}	44.13 ± 1.32^{def}
		DI	44.28 ± 1.08^{defg}	43.81 ± 2.03^{cdef}	47.55 ± 1.35^{hi}	46.76 ± 1.86^{h}
		DI + AMF	45.39 ± 1.78^{fgh}	46.39 ± 1.35^{gh}	49.16 ± 2.06^{ij}	50.29 ± 2.13^{j}
Total sugar content	10 DAP	WW	51.29 ± 1.83ac	48.6 ± 1.96^{b}	56.41 ± 2.23^{fg}	53.28 ± 1.98^{cde}
		WW + AMF	51.85 ± 1.76^{acd}	50.02 ± 2.07^{ab}	57.53 ± 1.76^{gh}	55.21 ± 1.67^{efg}
		DI	54.33 ± 2.21^{def}	52.22 ± 1.84^{acd}	60.19 ± 2.08^{hi}	57.86 ± 2.13^{gh}
		DI + AMF	55.33 ± 2.13^{efg}	55.52 ± 2.32^{efg}	61.47 ± 2.45^{i}	61.95 ± 2.55^{i}
	20 DAP	WW	66.19 ± 2.25^{ac}	62.91 ± 2.16^{b}	71.38 ± 2.96^{fg}	67.51 ± 2.63^{acd}
		WW + AMF	67.11 ± 1.57^{acd}	64.53 ± 1.57^{ab}	72.87 ± 1.78^{fgh}	69.67 ± 2.34^{def}
		DI	70.09 ± 2.84^{def}	67.86 ± 2.86^{cde}	76.12 ± 2.67^{hi}	74.09 ± 3.12^{gh}
		DI + AMF	70.92 ± 2.56^{efg}	71.82 ± 3.22^{fg}	78.46 ± 3.25^{i}	78.82 ± 3.55^{i}
	30 DAP	WW	80.48 ± 3.05^{ac}	76.09 ± 3.19^{b}	85.28 ± 3.64^{def}	81.12 ± 3.26^{ac}
		WW + AMF	81.42 ± 2.87^{acd}	78.03 ± 1.78^{ab}	86.92 ± 2.05^{efg}	83.56 ± 2.86^{cde}
		DI	85.28 ± 3.12^{def}	82.12 ± 3.82^{cd}	91.09 ± 2.79^{ghi}	89.21 ± 2.17^{fgh}
		DI +AMF	86.51 ± 2.09^{ef}	86.81 ± 4.13^{efg}	93.58 ± 3.86^{hi}	94.69 ± 4.12^{i}

$^{a–j}$ different letters are significantly different ($P < 0.05$) in each stage

DAP – days after pollination; WW – the well-watered; DI – the deficit irrigation; WW + AMF – well-watered and inoculated with arbuscular mycorrhizal fungi; DI + AMF – deficit irrigation and inoculated with arbuscular mycorrhizal fungi; 2X – the diploid watermelon; 2X/P – the diploid watermelon grafted onto the pumpkin; 3X – the triploid watermelon; 3X/P – the triploid watermelon grafted onto the pumpkin

could host a more abundant AMF and facilitated the interaction between the root and the AMF, which could enhance the efficiency in the uptake of the mineral nutrients and water. Mineral nutrition and water are essential for plant growth and are involved in virtually all metabolic and cellular functions. For example, Mg is an essential macronutrient that is required for important functions related to enzymatic activity, chlorophyll synthesis, CO_2 assimilation and phloem loading in leaves. The higher macronutrients concentration (N, P, K, Mg, etc.) and RWC in the watermelon line are beneficial for the photosynthesis and related enzyme activities, inducing the remobilisation of the assimilates from the vegetative tissues to the fruits with possible improvements in the fruit's quality and yield. Moreover, previous studies have reported that physiological differences are strongly correlated with gene expression changes in the host and some host genes are up-regulated or down-regulated by AMF (Silvestr et al. 2019; Tarnabi et al. 2020). Inoculated plants always maintain the exchange of substances between the AFM and the host, for example, small RNAs (sRNAs) can move across the contact surfaces from the AMF to the host, once in the host cells, they can regulate the gene expressions at the transcriptional or post-transcriptional levels in the host, through a mechanism known as RNA interference (Silvestr et al. 2019). In this study, the AMF effect on the RWC, P_N and enzyme activities of the pumpkin-

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root line was relatively high compared to the corresponding own-root line. It could imply that more substances favourable to yield accumulation and sugar synthesis from the AMF rootstocks move into the watermelon scion, and promote the expression of genes encoding the alkaline α-galactosidase, IAI, SPS and SuSy in fruits. The higher photosynthesis and activities of the alkaline α-galactosidase, IAI, SPS and SuSy would improve the fruit yield and sugar content in pumpkin-root lines.

CONCLUSION

Deficit irrigation significantly reduced the RWC, P_N, alkaline α-galactosidase activity, but increased the activities of IAI, SPS and SuSy during the fruit development stage in all the watermelon lines, which would reduce the synthesis of the photoassimilates, and improved the conversion of the photoassimilates to sucrose, led to a reduction in the fruit yield and an increase in the sugar content in the watermelon fruit. Although the AMF increased the RWC, P_N and activities of the alkaline α-galactosidase, IAI, SuSy and SPS under both the DI and WW conditions, the effect was more obvious under the DI condition. Meanwhile, the improvement magnitude of those parameters was greater in the pumpkin-root line than in the corresponding own-root line. As a result, the AMF in the DI grafted plants increased the fruit yield to a level similar to the WW values. The sucrose and total sugar contents were highest in the DI + AMF treatment among all the treatments, and the sucrose and total sugar contents in the grafted line were higher than those in the corresponding own-root line. The fruit yield and total sugar content were highest in the pumpkin-root triploid line among all the watermelon lines under the DI + AMF treatment. Our results suggest that an integrated application of AMF and DI to pumpkin-root watermelon plants is a promising approach to enhance the fruit sugar content with negligible yield penalties, especially in a pumpkin-root triploid line

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